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LA IMPORTANCIA DE ERIK LEONARD EKMAN EN LA EXPLORACIÓN DE LA FLORA DE CUBA

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(Llegado: 10 de Junio 1992)

The author analyzes the botanical career of the famous Swedish botanist, Erik Leonard EKMAN, based on his collecting activity interpreting his herbarium materials trying to find the professional and human motives of his long and insistent stay in Cuba and his highly enthusiastic dedication to the exploration of de Cuban flora, questions still unknown and not understood well.

Mi trabajo va a ser juzgado merecidamente después de cincuenta años, cuando ya no hay amigos ni enemigos, sólo la obra es, que se queda para hablar por mí.

Julián B. ACUÑA GALÉ

Esta oración del botánico cubano más grande y más modesto resueña en mi alma cuando estoy contemplando sobre la obra grandiosa y carrera fantástica de Erik Leonard EKMAN, uno de los exploradores más grandes de la historia universal de la botánica.

Ya pasaron más de sesenta años después de su fallecimiento temprano e inesperado, sin embargo todavía tenemos que hacer mucho para entender bien el tamaño y la importancia de su obra. No es suficiente que contemos el número de los géneros y especies que fueron colectados por la primera vez por él, que calculemos los miles de quilómetros que caminaba, los miles de metros que montaba. Hay que seguir sus trazos, subir lomas y picos que fueron conquistados por él, reabrir trillos en densidades que parecían intransitables antes de estar transitado por él, montar árboles y paredones, quemado por el sol y empapado por los aguaceros y saborear el sentido victorioso de descubrir un árbol grande, una palma curiosa o una flor bonita por nadie conocidos, los que en aquel momento nacen para la ciencia, como hizó él.

Gunnar SAMUELSSON (1931) y Tore HÅKANSSON (1986) dieron una reseña completa sobre las actividades, el aporte científico y del carácter humano

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de EKMAN. SAMUELSSON fue jefe y amigo de EKMAN durante su época humano de EKMAN. SAMUELSSON fue jefe y amigo de EKMAN durante su época de Española, conocía la carrera de EKMAN de sus cartas escasas y el mérito de su trabajo de la correspondencia con URBAN y otros especialistas del Museo de Berlin--Dahlem. HÅKANSSON diseñó un retrato de EKMAN más poético y literario con mayor enfasis al trabajo de los años cubanos utilizando datos obtenidos del diario de EKMAN.

Los necrólogos escritos por los amigos y por los rivales respectivamente (MOSCOSO, LEÓN, BRITTON), todos reconocen la grandeza de su obra. Sin embargo, en aquel momento nadie pudo estimar realmente la riqueza enorme y multifacética de aquel obra. No hay nada de admirar en esto. Para ver bien la forma y dimensiones de un rascacielos tenemos que oservarlo de mayor distancia. Asi mismo es con la obra de EKMAN también, que pasando los años sigue incrementando en tamaño e importancia.

En aquel momento, cunado murió EKMAN, todo el mundo vió en su persona el héroe romántico de la botánica, el explorador intrépido, el escalador de lomas, el colector y conocedor de plantas y al mismo tiempo vió el misántrope de carácter raro, y casi nadie dió cuenta al sabio multifacético de conocimientos muy profundos, al geógrafo, al fitogeógrafo, al fitotaxónomo excelente que fue especialista en las familias más críticas y difíciles de las Antillas, que hizo observaciones ecológicas fundamentales etc.

Muchos botánicos consideran que su trabajo efectuado en Española fue más valiosa, porque su papel en la exploración de aquella flora era indiscutiblemente fundamental, y la flora de la Española era mucho menos explorada que la de Cuba. Pero el valor de las dos exploraciones (en Cuba y en Española) no es igual. En Española EKMAN era el único botánico de gran talla, sin precursores notables, sin rivales contemporaneos, aunque las condiciones físicas del trabajo fueron más rigurosas que en Cuba.

La situación en Cuba era muy distinta. La flora bastante conocida, explorada por antecendentes famosos como Charles WRIGHT y John Adolph SHAFER, además estaba bajo un estudio intenso por un grupo de botánicos reconocidos, bien dotados, bien equipados. Todas estas circunstancias podían parecer muy atractivas a EKMAN para medirse con ellos, mostrar su preparación, probar sus calidades. La exploración de la flora de la Española parecía a una competencia donde el corredor compite solamente contra el cronometraje para obtener un nuevo record. La misma en Cuba parecía a un final olímpico, donde el corredor compite frente otros excelentes y para obtener un nuevo record había que ganarles también. Por esto — a mi juicio —, la

labor que realizó EKMAN en la exploración de la flora en Cuba fue más difícil, más grande y más importante de lo que cumplió en Española, a pesar de que esta última es una obra única e irrepetible también.

No creo que este muy lejos de la realidad al suponer este motivo como uno de los determinantes, que no dejaron a EKMAN durante muchos años que continuara su rumbo hacía Española. Todos que escribieron sobre la carrera de EKMAN no pudieron explicar su atracción tan fuerte a Cuba con otros motivos, solo enfatizando su carácter rebelde o insistente, que lo forzó para oponerse a los autoridades, quienes no encontraron las palabras adecuadas para influirlo. No dudo, que EKMAN se sentía ofendido por LINDMAN y el Comité de la beca cuando ellos no confiaron en su juicio y sus argumentos con los cuales pretendía convencerlos sobre la importancia y necesidad de una nueva exploración de la flora de Cuba. Sin embargo, este no podía ser la causa primaria de su permanecer en Cuba. En esta decisión -- que no era fácil —, debían jugar el papel más importante unos motivos científicos.

Para entender mejor estos motivos intentamos reanalizar los eventos conocidos del viaje de EKMAN complementandolos con los datos científicos que pudieron influir su decisión.

EKMAN partió de Estocolmo el 28 de Febrero de 1914, pasó por Lund para defender su tesis de doctorado sobre las Vernonias de las Antillas. "Al trasladarse a Cuba, pasó por Nueva York donde tenía oportunidad de visitar el Jardin Botánico y conocer a Nathaniel Lord BRITTON, el director del Jardin" -- según Alvarez CONDÉ. A mediados de Abril EKMAN llegó a La Habana. Allá se enteró de los intentos revolucionarios que habían empezado a brotar en La Española. Este junto con la aparición de la peste bubónica en la misma isla resultó que cancelaron los contactos directos por mar entre Cuba y Española de manera que EKMAN tuvo que quedarse en Cuba.

En aquel momento la flora de Cuba fue mejor conocida que la de La Española. El primer sumario de la flora fue publicado en la Historia Física, Política y Natural de la Isla de Cuba de Ramón de La Sagra, escrito por Achille Richard del Museo de Paris, incluyendo 1108 especies de plantas superiores. Como resultado de 10 años de exploración efectuado por Charles WRIGHT, este número se elevó a 3263 (GRISEBACH: Catalogus plantarum Cubensium, 1866) y con los complementos de WRIGHT publicados en la Flora Cubana de Francisco SAUVALLE (1873) a 3841 especies.

El mismo WRIGHT descubrió 22 géneros y 708 especies nuevos para la ciencia. Los años de la guerra de independencia de Cuba no favorecían a las investigaciones botánicas, pero desde los últimos años del siglo pasado una

actividad creciente se llevó a cabo efectuado por botánicos norteamericanos, como COMBS, CURTISS y más tarde por los diferentes grupos del Jardín Botánico de Nueva York, consistiendo de BRITTON y su esposa, la brióloga Elizabeth KNIGHT, además de Percy WILSON, COWELL, GAGER, HOWE, MERRILL y de John Adolph SHAFER. Este último, el más efectivo entre ellos y el primer explorador de algunas montañas limoníticas-serpentinosas (Sierras de Nipe y Moa) de Norte de Oriente. Estos grupos, junto con el Hermano LEÓN (Joseph J. SAUGET), profesor del colegio de La Salle de La Habana trabajaron muy efectivamente, descubrieron 8 géneros y 478 especies nuevos, elevando el número de las especies conocidas de la flora a unas 4300. Consideraban que con este número la flora de Cuba fuera bien conocida y BRITTON, WILSON y LEÓN empezaron la preparación de un catálogo de plantas cubanas con claves analíticas, que sirviera como base para la publicación de la obra Flora de Cuba. El manuscrito se confeccionaba entre 1914 y 1920 en 7 tomos encuadernados pero nunca llegó a publicarse en esta forma.

Al estar en Habana EKMAN hizo contacto con Hermano LEÓN, que orientó a EKMAN a lugares interesantes para botanizar y algunas excursiones hicieron juntos. Se supone (Alvarez CONDÉ 1957: 323) que "Urban diera instrucciones a EKMAN sobre visitar la Isla de Cuba antes de llegar a Santo Domingo, pues estaba interesado en obtener algunos datos sobre la flora cubana". Urban escribe claramente (Ark. Bot. 17 (7): 3, 1921, y Symb. Ant. 9: 55—176, 1923), que accordaron con EKMAN, que éste permaneciera por un corto rato en Cuba para conocer la variabilidad natural de las Vernonias antillanas y colectar más material para desarrollar su tesis de doctorado escrito sobre este género. Es probable, que URBAN aconsejó a EKMAN que utilizara su estancia para profundizar sus conocimientos sobre la flora antillana durante su espera al traslado a Española. Como se sabe, EKMAN en menos de dos meses colectó 1365 números de plantas. Estas fueron enviadas inmediatamente al profesor LINDMAN por encargo del profesor URBAN, quién encontró y describió algunas nuevas especies. Ya en estas primeras excursiones sorprendió a EKMAN la gran variedad de la flora; que areas muy cercanas, como la costa rocosa de Cojímar, las lomas calizas de Jaruco y los cayos serpentinosos de Guanabacoa, Loma de Coca y Canasí tienen florulas casi completamente distintas. Muy probable, que ya en estos primeros meses lo tocaron tres impresiones importantes:

1. La belleza y riqueza enorme de la flora.

2. Encontró que su preparación era en nada inferior a la de los demás botánicos que trabajaban en Cuba, y con buena práctica en corto período fuera capaz de desarrollarse a un especialista de la flora cubana.

3. En un país, donde en la cercanía de la capital se puede encontrar especies nuevas, la flora no se puede ser bien explorada.

Para disminuir los costos de vida EKMAN salió de Habana y se dirigió a la provincia Oriente, donde vivía una colonía escandinava en Bayate. Poco tiempo después de su llegada estalló la guerra mundial y la confusión general producido por ella se extendió a las Antillas también. "EKMAN estaba contento de poder estar allá y poder quedarse en un lugar en el que había encontrado muchos amigos entre los escandinavos que vivían ahí, quienes lo ayudaron de diferentes maneras" — como escribe SAMUELSSON. Sin embargo, sobre todas estas ventajas, Bayate debía influir decisivamente la carrera de EKMAN, por su posición geográfica y fitogeográfica particular.

Bayate se encuentra en la embocadura del Valle Central intramontano de Oriente cerca del pié sur de la Sierra de Nipe. De poca distancia al Norte se elevan los farallones calizos de la Sierra de Nipe, que bordean el Altiplano serpentinoso de esta vieja montaña, con el mogote grande aislado del Picote en la cerquita, mientras al Sur se ven los cienes de cumbres azulosos de la cordillera larguísima de la Sierra Maestra. Areas inmensas virgenes, la mayoría de ellas completamente inexploradas. EKMAN en aquel momento tenía tiempo para explorarlas y lo hizo.

Bayate se halla en el punto donde se encuentran las florulas de tres sectores fitogeográficos riquísimos de Cuba: la flora de la llanura centro-oriental de Cuba, la del Macizo de Sagua-Baracoa y la de la Sierra Maestra. Este lugar desde el punto de vista florístico es igual favorable, que el Monte Verde, donde Charles WRIGHT empezó sus exploraciones 60 años anterior.

EKMAN exploró primero las sabanas alrededores de Bayate, luego escaló al Picote de Miranda y después a los farallones y encontró una flora completamente distinta de la que había conocido de Habana. Luego penetró a la zona serpentinosa de la Sierra de Nipe, subió por el Paso Estancia a la Loma Estrella, llegó a La Mensura, la altura más eleveda de la montaña, bajó en el valle fabuloso del Rio Piloto que era un Eden para botánicos.

Observó que subiendo por el Sur a la montaña se encontró una flora distinta de la que había explorado por SHAFER escalando por el Norte hace algunos años. Además, los valles de los ríos saliendo de la meseta en distintas direcciones, también tenían sus florulas própias diferentes caracterizadas por especies endémicas locales de los géneros _Tabebuia_, _Psidium_, _Eugenia_, _Phyllanthus_, _Leucocroton_. Le chocó la tremenda diversidad de esta flora. Colectó docenes de especies nuevas, y un par de géneros nuevos, como _Ariadne_ y _Harnackia_. Los éxitos de estas primeras excursiones orientales

seguramente fortalecieron su impresión que la flora de Cuba de ninguna manera era tan bién conocida como se suponia en aquel tiempo.

Siempre lo atraeron las alturas. No pudo resistir por mucho tiempo a la tentación de conquistar la cima más alta de Cuba, el Pico Turquino. Después de un intento inexitoso, en 17 y 18 de Abril 1915 logró a llegar al cumbre del Turquino, acompañado por su amigo sueco J. A. NYSTROEM y por dos prácticos cubanos de Nagua, Regino VERDECIA y Joaquin "Prerrucho" RODRIGUEZ. Aunque más tarde se aclaró, que Fred W. RAMSDEN fue el primero, quien escaló al Pico Turquino en 1860 por el lado Sur, esta subida no tenía significancia particular por no haber producido resultado científico. Por esto, la primera conquista del Pico Turquino de valor científico fue realizado por EKMAN, quién nombró los picos desconocidos, en el Estribo Norte del Turquino (Loma Regino y Loma Joaquin por los nombres de los prácticos) y las cimas laterales del cumbre principal del Pico Turquino (Pico Cuba 1862 m, y Pico Suecia 1734 m) por el nombre del país anfitrión y lo de los visitantes. Midió la altura del Pico Turquino e hicieron una exploración botánica muy valiosa descubriendo el género endémico de la Sierra Maestra (Solonia, Myrsinaceae) y colectaron 29 especies todos nuevos para la ciencia.

Tore HÅKANSSON (1986) dió el título a su excelente artículo sobre EKMAN: "El botanista sueco en el Pico Suecia". En la realidad, EKMAN nunca logró a llegar a los picos laterales del Turquino, porque subiendo del lado Norte estos picos se quedan lejanos y ni en la primera ni en la segunda subida tenía tiempo para explorar los picos mencionados. En favor de esta afirmación podemos referirnos al hecho, que en las colecciones de EKMAN faltan algunas plantas muy comunes de estos picos, como Ilex turquinensis, Ilex nannophylla, Chaptalia turquinensis, Scolosanthus maestrensis, Mitracarpus acunae, que fueron descubiertas más tarde por otros botánicos (ACUÑA, ALAIN, BORHIDI y MUÑIZ, etc.). Todo el mundo quien sube por el lado Sur debe llegar pasando por el Pico Cuba, así esta cima es bastante frecuentada. Sin embargo, el Pico Suecia se encuentra al termino de una estribación Suroriental de Pico Turquino que se queda lejana de los trillos practicados comunmente. La primera expedición que conquistó el Pico Suecia fue organizada y dirigida por el famoso geógrafo cubano, Antonio NUÑEZ JIMENEZ, en octubre de 1945. La segunda expedición fue realizada por botánicos y zoólogos cubanos bajo la dirección de Onaney MUÑIZ e Israel GARCÍA, en diciembre de 1969 con la participación del autor de este articulo. Esta vez

se efectuó la primera exploración botánica del Pico Suecia (véase: BORHIDI, 1974).

SAMUELSSON escribe (1931), que "Durante la primera parte de su estadía en Cuba, EKMAN no tenía, desde luego, un gran conocimiento de las especies que existían allá. Tampoco existía en Cuba ningun resumen acerca de las especies existentes. Una buena base para sus investigaciones fueron las listas que el profesor URBAN había preparado. Poco a poco iba conociendo las plantas y podía decir si las había visto antes o no. Es por eso que inclusive sus colecciones cubanas contienen muy pocas de las especies más comunes."

Mi impresión es, que EKMAN se había profundizado mucho más rápidamente en el conocimiento de la flora cubana que lo era considerado. Estudiando sus primeras colecciones tenemos que prestar una atención especial a algunas cifras. Como mencionamos, durante los primeros dos meses de su estancia en Cuba, EKMAN colectó 1365 números de plantas en el alrededor de Habana, una area bien conocida, sobrecolectada. Sin embargo, EKMAN encontró 12 especies nuevas para la ciencia. Este número de hallazgos nuevos no lo tenemos que considerar un resultado sobresaliente en cualquier otra parte de Cuba, sin embargo, en aquella area habanera este aporte — obtenido además, por un botánico inexperimentado en Cuba — debe ser cualificado como notable. Ya en este primer período tenemos que fijarnos a un fenómeno relacionado a sus colectas: que casi no hay plantas comunes repetidamente colectadas. Para uno, que no tiene conocimientos bastante confiables acerca de la flora — teniendo en cuenta la variabilidad enorme de algunas especies muy comunes (como Eugenia foetida, E. confusa, Cordia gerascanthus, Tabebuia myrtifolia, etc.) -- es completamente imposíble colectar sin repeticiones.

Al llegar a Oriente, una zona mucho menos explorada, sus colectas se incrementaron en novedades. Desde el junio de 1914 hasta el fin de abril 1915, en 10 meses coleccionó unos 4200 números más, que contenían nada menos de 5 géneros nuevos y 285 especies nuevas. Esta efectividad aproximó a la de SHAFER, el explorador más reconocido de los años anteriores. EKMAN reconoció que la flora era extremadamente rica en zonas donde areas de calizas y de serpentinas se encontraban juntas o sobrepuestas, como lo vio en la Sierra de Nipe. Empezó a buscar lugares de este tipo y los encontró en el alrededor del Yunque de Baracoa, en la Bahía de Taco y en varios valles de la Sierra de Nipe, sobre todo en el valle del Rio Piloto, que es la localidad clásica de muchísimos tipos. En la area de Baracoa colectó un género y 61 especies nuevos; en Taco y Maraví otro dos géneros y 47 especies nuevos más, en la

Sierra de Nipe dos géneros y 109 especies nuevos, en el Pico Turquino un género nuevo y 25 especies nuevas, nada menos de 6 géneros nuevos y 242 especies en total. A veces occurrió que entre 8 números colectados tenía 4 especies nuevas, además entre 4 números todos resultaron especies nuevas!. Lista impresionante para un principiante!

Para ilustrar la eficiencia de EKMAN presentamos dos ejemplos de la libreta de EKMAN:

Tabla 1

Detalles de la libreta de campo de E. L. EKMAN

4027	Psidium araneosum Urb. sp. n.
4028	Schmidtottia monantha Urb. sp. n. y género nuevo.
4029	Jacquinia obovata Urb. sp. n.
4030	Buxus obovata Urb. sp. n.

4034	Acrosynanthus minor Urb. sp. n.

4039	Sarcomphalus bidens Urb. sp. n.
4416	Calyptranthes apoda Urb. sp. n.
4417	Ilex ekmaniana O. E. Schulz sp. n.

4422	Schmidtottia multiflora Urb. sp. n.
4423	Clusia monocarpa Urb. sp. n.

Es característico para la ambición y autocrítica rigurosa de EKMAN, que se cualificó más tarde "que era como un niño cuando llegó a Cuba" -- según una carta de LINDENIE dirigida a LINDMAN (SAMUELSSON 1931), el 3 de Mayo 1920. Sin embargo, sus colecciones de aquel época temprana dicen, que él reconoció muy rápidamente los géneros más ricos y variados de la flora, como Psidium, Calyptranthes, Eugenia, Miconia, Ossaea, Phyllanthus, Tabebuia, Buxus, Rondeletia, etc. — y empezó a colectarlos sistemáticamente.

EKMAN reconoció que la mayoría de las plantas florece y fructifica en la época de lluvias -- sobre todo en Oriente — una estación poco frecuentada por los colectores americanos, que preferían la época seca para sus expediciones. En esta forma, las colectas de EKMAN resultaron más eficientes y muchas especies conocidas hasta entonces solo en ejemplares estériles fueron colectadas por la primera vez en ejemplares completos por él.

Esto es el motivo, porque el volvió a colectar las mismas zonas dos-tres veces en distintas estaciones hasta que logró completar sus colecciones con ejemplares flor- y fructificados, que permitieron su descripción correcta. Gracias a la insistencia concienzuda de EKMAN, URBAN tenía que

basarse muy pocas veces en materiales incompletos, cuando describió nuevos taxa, además, estas colecciones completas y abundantes ayudaron a URBAN que reconociera un número considerable de géneros nuevos, como Ariadne, Acrosynanthus, Auerodendron, Doerpfeldia, Krokia, Mozartia, Myrtekmania, Ottoschmidtia, Reynosia, Schmidtottia, Siemensia, etc.

Para perfeccionarse en conocer las plantas cubanas EKMAN consiguió permiso de estudiar el material del Herbario Sauvalle. Esta colección de importancia fundamental tenía una serie casi completa de duplicados de las plantas colectadas por Charles WRIGHT entre 1856 y 1866. Este material valiosísimo se encontraba entonces en la Academia de Ciencias Médicas de Cuba en Habana. EKMAN tenía acceso de estudiarlo antes de Hermano LEÓN, quién publicó su reporte sobre el Herbario Sauvalle solamente en 1939, 15 años después de la salida definitiva de EKMAN de Cuba.

EKMAN no solo estudiaba el Herbario Sauvalle, sino fue él el primero al revisar críticamente esta colección dejando notas de lapiz con su caligrafía de letras redondas características, cuando corrigió determinaciones erroneas o nombres inválidos. Estas notas nos muestran a EKMAN, como el mejor conocedor de la flora de Cuba. Este rango, que él logró a los años 1919—1920, fue reconocido también por los representantes contemporáneos de la ciencia cubana, cuando lo eligieron miembro de la Sociedad Cubana de la Geografía. Para esta fecha llegaron a Europa los materiales de sus colectas realizadas entre 1915 y 1919 — demorando varios años por la guerra mundial -- que resultaron sorpresivamente valiosas y ricas en novedades y justificaron su decisión de haber quedado y trabajado tantos años en Cuba. Su prestigio científico se había aumentado y fue ampliamente reconocido.

Una historia refleja en forma característica a sus conocimientos profundos. Una vez, Hermano LEÓN dió un discurso en la Sociedad Cubana de la Geografía donde hizo conocido los resultados botánicos de sus exploraciones hechas en las zonas serpentinosas de Cuba Central. Entre varias novedades presentó una especie nueva descrita y nombrada por BRITTON y WILSON como Psidium Loustalotii de la familia Myrtaceae, dedicado a un colega quién acompañó a Hermano LEÓN a esa expedición. Terminada la presentación EKMAN investigó el ejemplar y dijó la sentencia cruda: "No es nueva, ni pertenece al dicho género, ni a la familia tampoco." Y tenía razón, porqué la planta no era otro, sino un ejemplar de la Guettarda echinodendron de la familia Rubiaceae, descrita por Charles WRIGHT 50 años antes.

La insistencia en el trabajo y la resistencia frente las incomodidades, dificultades en las condiciones físicas, alimenticias, etc. son las

características de EKMAN mencionadas por todos sus biógrafos. De la fotografía famosa de Ekman -- publicada varias veces en distintas obras -- podemos formar una idea, como trabajaba él en el campo, esta "expedición unipersonal", con una prensa con plantas sobre el pecho, otra con papeles de periódico secos sobre su hombro y un machete en la mano para abrir trillos; este fue todo su equipo.

Una vez, en la Sociedad Cubana de la Geografía fue EKMAN el conferenciante, y presentó los resultados de una exploración aventurosa que hizo en la Sierra de los Organos, escalando paredones calizos de mogotes inaccesibles. Acabando el discurso, una señora bien vestida del auditorio preguntó, que si estas expediciones no estuviesen demasiado costosas. — "Puede ser, señora", — contestó EKMAN con una serenidad divina, — "sabe Usted, cuantos pesos había tenido en el bolso al salir de Habana? Tres. Y sabe cuanto tenía, cuando regresé? Tres también. Así son costosas mis expediciones."

Su situación económica era la pésima luego de su primera estancia en Haiti, cuando volvió hacía Santiago de Cuba, sin dinero. Su intento anterior para encontrar alguna subvención del Jardín Botánico de Nueva York, fracasó. Lo ayudó en aquel momento el Museo Nacional del Smithsonian Institution que le ofreció una modesta subvención por colectar gramíneas para la Exsiccata de "West Indian Grasses" editada por A. S. HITCHCOCK and Agnes CHASE, especialistas destacados de aquel instituto, que reconocieron el agrostólogo de capacidad extraordinaria en EKMAN, y lo emplearon en su proyecto. Varias cartas de Agnes CHASE dirigidas a EKMAN conservadas en el archivo del Instituto de Ecología y Sistemática de la Academia de Ciencias de Cuba, confesan sobre una relación colegial y sinceramente amistosa que desarrolló entre ellos.

Para colectar hierbas, EKMAN tenía que recorrer las areas sabanosas de toda Cuba incluso la Isla de Pinos, pero los viajes eran costosos. EKMAN visitó al director general de la Companía Cubana de Ferrocariles y ofreció que iría a dedicar unas plantas nuevas a su honor si subvencionara las expediciones. EKMAN recibió un pase de circulación gratuito, válido para todas las lineas ferrocariles de Cuba, y un cierto señor REED recibió unas plantas nominadas para él, como <u>Andropogon reedii</u>, <u>Barleriola reedii</u>, etc.

EKMAN resultó muy exitoso en sus exploraciones agrostológicas. Descubrió 3 géneros nuevos y 14 especies nuevas que permitieron crear algunas combinaciones nomenclatóricas sumamente raras. Por ejemplo en un género nuevo descrito por él -- con la co-autoridad de Agnes CHASE -- y dedicado a su rival, Hermano LEÓN (<u>Saugetia</u> Ekman & Chase) la segunda especies fue

dedicada al autor del género (Saugetia ekmanii). En otro caso logró a describir una especie nueva perteneciente a un género dedicado anteriormente a él (Ekmanochloa aristata Ekman).

Cada vez, cuando trabajo con las plantas colectadas por EKMAN -- conociendo también las condiciones en que trabajaba él -- siempre me maravilla el estado impecable de sus especímenes de herbario prensados y secados con una perfección ejemplar. Dicen que el secreto radica en lo que EKMAN, luego de haber colectado durante 3-4 días entre las lomas, dirigió sus pasos a una aldea, donde había una panedería. Por la noche al llegar a la panadería, EKMAN pidió permiso para poner sus prensas en la puerta de la estufa y toda la noche vigilaba, que las prensas no cojieran candela por el calor de la estufa. Al amanecer dió gracias y volvió a la montaña.

Estando en el campo EKMAN vivía sumamente barato. No llevó otro por encima, sino una cantinplora de té y unas gelleticas. Si por su camino llegó a algunas fincas aisladas en el monte, los campesinos lo invitaron a comer con ellos lo que había, platano hervido, boniato y a veces un pedacito de carne asada del cerdo caserio o de la jutía conga, que capturaron los prácticos en las lomas.

EKMAN tenía muy buena relación con los campesinos. Ellos apreciaron mucho al hombre sabio y modesto, que conocia tantas cosas sobre la naturaleza de Cuba. Los campesinos Le ayudaron mucho. Se lo mostraron los trillos secretos de los jutieros que llegaron a las cimas de las montañas, se lo explicaron el nombre vernacular de muchas plantas colectadas por EKMAN y los usos, como ellos las aplican. De ellos colectó EKMAN los nombres vulgares de los tipos de vegetación, que introdujó en la literature fitogeográfica: los terminos de "yayales, manacales, fangales, cuabales, charrascales y de monte fresco", que aparecieron por la primera vez en el tomo 9. del Symbolae Antillanae en la interpretación de Ignatius URBAN.

No creo, que me fellese mucho al suponer, que este ambiente rico de la naturaleza y rico en humanidad pura fue uno decisivo entre los motivos que no lo dejaron a EKMAN que saliera de Cuba durante tantos años.

El otro motivo fuerte es -- sin duda alguna -- que EKMAN era un gran competitor. Ya hemos visto que dentro de pocos años acumulaba tantos conocimientos sobre la flora y vegetación de Cuba, como tal vez nadie en aquel tiempo, y él quisó disfrutar estos conocimientos hasta mayor profundidad. Él era mucho más que un colector profesional sobresaliente, era un botánico y geógrafo de gran talla, que no solamente conocía las plantas, sino conocía sus habitades, sus distribuciones, sus requerimientos ecológicos. Es muy

seguro, que él pretendía, o por lo menos pensaba escribir un trabajo sobre la fitogeografía de Cuba, como lo fue prelegado por URBAN (1923). Aunque no estuviese ni declarado ni confesado, se desarrolló un concurso en la exploración de la flora de Cuba entre Nueva York y Berlin, y EKMAN ere "la punta de lanza" de las aspiraciones alemanas. Hay que enfatizar, que esta situación no desarrolló intencionalmente. Ni URBAN, ni EKMAN, ni el Museo de Estocolmo quizó meterse — originalmente — en el descrubrimiento de la flora cubana. Las emociones estallaron en julio de 1922, cuando Hermano LEÓN y dos entomólogos americanos, Charles BAILLOU y Stephen BRUNER hicieron una expedición al Pico Turquino con el concurso de EKMAN, el que sirvió como el guia de la excursión. Hermano LEÓN escribió un articulo muy detallado sobre la historia, las colectas y los resultados de la excursión, sin hacer mención alguna sobre algunas discusiones amargadas entre EKMAN y él sobre la prioridad de colectar algunas plantas raras, casos que occurrieron algunas veces durante las dos semanas de la excursión. Sobre un mayor incidente entre ellos hay una anécdota descrita por Alvarez CONDÉ (l.c. 325—326) refiriendose a Julián ACUÑA, el que conoció el caso del mismo BRUNER.

"Subiendo por el Rio Yara cargados hasta el límite con mucha agua y marcha fuerte, hubo un momento en que el Hermano LEÓN se agotó y pidió descansar. EKMAN, que era un hombre muy impulsivo regresó y arrebatandole la carga al Hermano LEÓN dijó: — Niño, cuando los hombres no se encuentran en condición tales, no debían intentar empresas que sólo son de hombres. — Esta era la táctica que EKMAN usaba para llamar al amor propio y poder terminar su empeño. Ya antes EKMAN había estado disminuyendo la capacidad de los norteamericanos y destacando la alemana y el vigor de los hombres de su país."

En la versión, que yo oí de ACUÑA, entre los "niños" EKMAN mencionó a los franceses también, refiriendose a la nacionalidad original de Hermano LEÓN. Parece que aquellos años de la guerra mundial, — cuyas consecuencias hasta ahora siguen sufriendo los pueblos de Europa, — no hayan pasado sin dejar trazos en el alma de la gente, — por lo menos de los europeos — que vivieron en las Antillas. Sin embargo, — a mi juicio — esta anécdota no es tan característica ni tan importante, como el aporte incomparable de EKMAN a la exploración de la flora de Cuba, que no esta tratada merecidamente en la obra citada. Tal vez, por que el autor era un alumno de Hermano LEÓN.

La que caracteriza al EKMAN verdadero para mí, es otra anécdota. Él mismo escribe al profesor LINDMAN en 1923, lo siguiente: "...durante una de mis últimas excursiones tuvé mala suerte de caer desde un árbol de 10

metros de altura, a consecuencia de lo cual me rompí la muñeca y un par de costillas."

Sobre este accidente de EKMAN hay un anécdota. A los fines del año 1922 EKMAN hizo una excursión para Nagua, la parte occidental de la Sierra Maestra. Salió de Bayamo y siguió el valle del Rio Yara. Llegó a un estrecho del valle, donde vio un arbusto florecido sobre un farallón en unos 10 metros de altura. Supusó que este arbusto fuera una Rondeletia desconocida. Lo que conoce este género muy complejo de la familia Rubiaceae, cuyas especies se distinguen en diferencias minuciosas, muy poco aparentes, puede estimar la intuición y los conocimientos profundos de EKMAN. Por una planta común seguramente no habría riesgado el peligro de matarse. Pues, el farallón no era accesible para subir, EKMAN montó a un árbol cercano y desde la copa del arbolito intentó alcanzar la planta deseada. Al momento de alcanzarla la rama del árbol se rompió, EKMAN se cayó y se le rompió la muñeca. Duraba dos días que EKMAN llegó con sus colecciones y brazo roto a Bayamo, donde en el hospital lo atendieron. Cuando se recuperó volvió al farallón e intentó la subida por la segunda vez. Se cayó de nuevo, se le rompió un par de costillas y se hirió el otro brazo. Volvió a Bayamo donde dejó curarse otra vez. Pero después de tres semanas estaba de nuevo al pié del farallón fatal y por esta tercera vez logró a colectar la planta anhelada, que por supuesto, resulto ser nueva. Fue descrito por STANDLEY, y la especie nueva que costó tanto sufrimiento a EKMAN que ninguna otra, fue dedicada al honor de él recibiendo el nombre: Rondeletia Ekmanii.

Aunque todos los elementos de este cuento son de verdad, el cuento completo parece una fábula. Como han pasado los años, EKMAN, el botánico solitario, el escalador de lomas, el botánico intrépido se ha convertido en un héroe de la ciencia, héroe de cuentos fascinantes, el botánico heroico. Era una figura romántica cuyo ejemplo nos hace palpitar mas rápido el corazón — también en nuestros años deheroizantes — y nos hace creer, que empeño, dedicación y misión son tres ideas por las cuales es una tarea digna de vivir.

En los últimos tres años que llevó en Cuba, hizo un labor formidable. Recolectó de nuevo toda la isla. Sus colectas hechas en esta época son las más eficientes, más valiosas. La mayoría de los tipos se colectó en estos tres años. Y se despedía. Ya supó que su traslado a Española era definitivamente decidida. Se despedía pues, de las lomas superbas, de los farallones peligrosos, de los valles ricos y del paisaje de los palmares harmónico y encantador. Porque Cuba era el gran amor: su juventud. Esta nostalgía suena en una de sus cartas:

"He sacrificado mi vida por el conocimiento de la flora de Cuba. He pasado hambres, sufrimientos y enfermedades que me han puesto muy cerca a la muerte." — ¿Pero porqué? — podemos preguntar. Nadie quizo que lo hubiera hecho. Este es el amor. El amor a la flora de Cuba.

Ya estando en Haití sigue teniendo mucho regaño por Cuba. En sus cartas repite a menudo el deseo de poder volver para poder terminar su trabajo allá. En octubre de 1925 escribe a URBAN: "¡La flora que de Cuba podría escribirse! Qué lástima, que no pudé ir por la zona de Baracoa una vez más. Me gustaría pasar un año (!) en esta parte de Cuba y estoy seguro que la botánica me beneficiaría enormemente con esto." Este es nada menos que un grito de socorro, del anhelo por el amor prohibido, por el paraíso perdido, por el sueño mil veces soñado. Quizás, EKMAN sospechó algo de lo que sus aspiraciones sobre la publicación de un trabajo grande sobre la flora y vegetación de Cuba no iba a realizarse jamás.

Sin embargo, la flora de Española lo consolaba y compensaba de manera espléndida por los descubrimientos dejados en Cuba, pero los heridos de alma lo torturaban todavía por un buen rato.

Tabla 2

Cifras interesantes sobre el trabajo colector de EKMAN

	Cuba	Hispaniola	Total
Números de plantas colectadas	19,251	15,463	34,714
Géneros nuevos descubiertos	31	15	46
Espécies nuevas descubiertas	910	969	1,879
Eficiencia de descubrimiento			
Ejemplares/género nuevo	621	1,030	760
Ejemplares/especies nuevas	21	16	19

Las cifras de la tabla 2. dan una idea sobre el tamaño del labor de EKMAN y sobre la eficacia tremenda de su actividad coleccionista. En Cuba durante 9 años colectó 3788 números más que en Española durante 8 años. El hecho, que la flora de Cuba era mejor explorada que la misma de la Española esta indicada por el número menor de las especies nuevas descubiertas en Cuba. Pero la mayor diversidad de la flora de Cuba se refleja en el mayor número de los géneros descubiertos. En cuanto a la efectividad colector de EKMAN podemos ver, que cada 21 ejemplares colectados contenían una especies nueva en Cuba, en promedio, mientras en Española su eficiencia resultaba todavía mayor alcanzando una nueva especie en cada 16 ejemplares colectados.

Con este aporte EKMAN logró a convertirse en uno de los colectores más exitosos y más eficientes no solo en las antillas, sino en la historia general de la botánica también.

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PLAY TO BE SAFE.
AN INTUITIVE MEDITATION ON THE SCIENCE OF ECOLOGY

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"Experience teaches us that something is such and such, but it does not point out that it cannot be anything else."

(Kant, I.: The Criticism of Pure Sense, 1787.)

In the frame of a conceptual and historical looking back an attempt has been made by the author to explain a new possible approach of the science of ecology. The main praeambles and conclusions were as follows: — most of the concepts of ecology are deeply impregnated with the Hobbesian slogan: "War of each against all". — Darwin's ideas have been highly distorted by his "followers", and there no an adequate analysis can be found about his concepts from ecological point of view. — We know nearly nothing about the history of ecological thinking. — According to the author's view; communities are fundamentally non-hierarchic entities. — Striving for safety; an universal eco-genetical behaviour, which concerns every organism, and is realised on the range of species.

"Oikos"

The word ecology originates from the Greek word "oikos" which means house, place of residence, household, and in a wider sense, surroundings... A great many general notions, the meaning and scope of which is pretty elastic, either a very simple meaning is hidden in them, or they refer to terribly complicated, illimitable profoundness and interconnection. One thing is sure: these notions include the necessity of coexistence, if it is better this way, they include all the visible, perceptible and latent marks of their coenological or "sociological" coherence, just like as they show their dependence on each other in the sense of biocoenology.

Disregarding all the above-mentioned signs the definition of ecology cannot be interpreted at all, but the question is as follows, knowing all this, from what aspect do we want to take snapshots from the enormous variety and multidimensional networks of the living world?

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Ecology: "War of each against all"?

The word ecology first was used in today's meaning by Ernst HAECKEL in 1869, expressing relations between the societies of creatures and their surroundings. We should not think by any means that the thinking bearing ecological content and the history of ecology, at all, is registered from the before-mentioned date. WORSTER (1984) and McINTOSH (1985) who — no doubt -- have been peerless in the thorough researches of the history of ecology, even they do not stick to this date, atlhough WORSTER is a little shy in this sense, when, with the illustrious figure of Gilbert WHITE, he almost hides the long past.

More than a hundred years have passed since then, and these years have brought about a lot of aspects how to approach ecology, and a lot of definitions, as well from among these, great many have not ceased fighting one another (e.g. the opposition of the conceptions of autecology and of synecology etc.). The most striking about the battles is that not only authors and theories disagree with one another, but — rather strangely — plants and animals, their populations and their communities have become perpetrators of brutal violence. According to the prevailing opinion of our times: harmful and useful creatures, aggressive plants and animals fight one another, or they simply endure one another for a short time. Most of the phenomena — having an "oikos" character -- are hidden because causing a recent-time war, of the law of "competitive exclusion", moreover, we will never be able to get to the truth, to get deep into the sciences of ecology and of biocoenology. We will never be able to understand the concatenation of creatures from the behaviour habits which are summed up in indexes and differential equations. Neither would mathematics help us with its descriptive character. It is impossible that all the mathematicians who became "experts" at ecology have forgotten one of Newton's famous sayings that he announced when he was asked about the reasons of gravitational laws in connection with the movement of planets. He said that the law "describes how the planets move...but it does not tell why!" (In FEYNMANN 1965.) It must become obvious that the question of "why" can get a more or less adequate answer from a biologist who applies a bio-methodology that would in every sense comply with the examined objects. One of the conclusions drawn from the works of ODUM, HARPER, GRIME, WHITTAKER and GAUSE — to mention only a few from among the well-known ecologists —, unfortunately, is, as follows: there is a struggle between the organisms and their associations, the compe-

tition -- as DARWIN conceived it ("struggle for existence") -- is at its peak. This sort of observation leaves strong ripples even in the conceptional stock of "coevolution", to say nothing about the insect-plant relationships (e.g. Ecology 1988 December, or FUTUYMA and SLATKIN 1983 etc.).

Knowing DARWIN's works, and entering fully into the spirit of his works, I must announce that he did not regard the "battle of life" as tragic and sharp as the ecologists of our century do. According to DARWIN the single organisms of the species and their communities do not take up only battles but they dance their "wedding dance" as well, they accomplish their life-cycles, create their "home" and "household", in short, -- in the first place -- they try to make an attempt for living, just like their fellow sufferers do. The young researcher (travelling on board of H.M.S. "Beagle" -- while writing a diary) brought together an ingenious collection of examples on the behaviour of creatures, and all this he had to do without having any previous knowledge in this particular profession and neither in terminology.

There was no other opportunity to express his thoughts concerning different sorts of behaviour than the terminology which was accepted by the human society. That is why the appearance of such notions like aggressiveness, war etc. in the characterization of different creatures is not just a mere chance. In this way DARWIN can be presented in much more aggressive colours than he really was. Unfortunately, here again crops up -- from behind the misinterpretations -- the lack of historical knowledge. It is a hardly ever mentioned fact again, regardless that MUMFORD (1960--1973) explicated in several of his works, that is was T. H. HUXLEY, "Saint Paul of Darwinism" who turned Darwinism into the ideology "of the blood-toothed and blood-clawed Nature". Unfortunately, this opinion predominated over DARWIN's deeper view of life, for a pretty long time it distorted the slowly unfolding, organic world concept. We can say that this opinion -- even now -- acts as a distorting factor on the world concept.

DARWIN, several times, "has come to grief", because in several sciences -- in ever so many cases --, incorrectly, he has been referred to as their establisher, ever so striking it is that, primarily, he was an ethologist, an animal ethologist, and it is known for all who have read his works.

Nevertheless an ethologist would have a need to come across ecology which is in the thinking of having an ecological aspect, he would come across the science of associations, plant and animal geography, and anatomy, even if he cannot or does not want to accept it. It would never enter into

our minds to call Jean Henry FABRE a phytocoenologist or a botanist, although we can find excellent biotop-like, ecological and floristic descriptions in his ten-volumed work titled "Souvenirs Entomologiques".

From this point of view WALLACE might consider himself luckier than DARWIN. Although WALLACE became less famous than DARWIN, but not so many labels have been attached to his name, and so, the opinion formed about him is more clear-cut.

The prevailing "arrangement" in this history of science is, naturally, influenced by the paradigms more in usage. Towards the end of our century ecology including classical ethological characteristics, and ecology observing and/or describing that flora and fauna have lost ground, or it has indeed become latent. The greatest disadvantages of this can be detected in that, that we almost imperceptibly become estranged from the beings of nature, and while it is happening, the real life-cycles and strategies remain concealed from us. Owing to this phalansterian view — among the others — some of the cologists, as time has passed, have become orientated in incalculable ecosystem horizons. From their studies — slowly but surely -- reality was excluded. The capability of the actual studying of reality has shrunk to the level of illusion.

The ecosystem avalanche was started by the article of ODUM (1969) -- the article became famous —, and the snappiest answer, up till now, has been given to it by JUHÁSZ-NAGY (1985). Besides the before-mentioned contrastudy there crops up the question as well: how could they extract the plant and animal associations, or synusia etc. from the ecosystem? There is no doubt that they became martyrs of a mechanical mass, since what else could be ecosystem than a man-constructed, mechanical, inconceivable "time machine" which cannot be studied directly. The following question lends itself to be asked: are the long studies written on vegetation deemed to damnation?

If we just take a look at the most widespread definition of ecology in our times -- it is from the book of SZÉKY (1983) — it reads: "Ecology is a science which analyses — on a methodical basis -- the interrelation system between the creatures and their surroundings, using the relating knowledge of physics, chemistry and biology. It combines the interrelations of sciences (interdisciplinary). The basic unit of its research most of the time is the ecosystem, in which the surroundings — with bio-, phytocoenological elements in and on it — creates and keips up a close interrelation system ... etc...." Let us see the definition of ODUM's (1963): "... ecology is concerned especially with the biology of groups of organisms (Which groups?)

and with functional processes on the lands, in the oceans, and in fresh waters (Which functions and/or processes ???), it is more in keeping with the modern emphasis (Modern ? — No comment...) to define ecology as the study of the structure and function of nature". This is a behaviour called "...to forget the past and exalt the present..." (WORSTER 1984).

Chemistry, physics, interdisciplinary, interrelation system and eco-system, structure, function, fundamental unit of examination... Definitions speak for themselves: there is something wrong here. Here crops up the question as well: are there any groups of researchers who can get even a step forward in such a chaotic branch of knowledge? I think that every researcher would call it a day. Where, in all this, is the definition the logic one? The most dangerous about the definition is that it has got a too strong "inter"-characteristic, the doubt, with good reason, can come up in the mind of the more sober-minded researcher from whom a well-meant interference is not alien either. Well, and who is the person with his crystal-clear logic who wants to set the things right first? Nobody else: but a mathematician! And on this point the knowledge of the person knowing "oikos" comes to an end... The spiritless, foreign method with its iron logic that is mathematics is forced upon biology which has an endless storehouse of behaviour, and it necessarily turns the living world into something which is atomical. Mathematics direct the interrelation woven of milliards of threads during which quality disappears. There now! We have fallen from the high-flown level of ecosystem to the level of the atoms and elements, to the dimension where is structure but not life!

At least we must put up the question: how long can we keep up the attitude in trying to get to know the fundamental phenomena of life (taking into consideration either the cells or the associations) using only sciences which have a descriptive character? It would be possible, only then if the creatures were "automatic machines". Of course it has been a tempting dream that certain interrelations can be described. Moreover they can even be explained on the level of the quality of life, but only if we use mathematical or even physical methods and laws. For example simultaneous equations. In this aspect of description and statistics, it is possible. It is no worth arguing about it, but as an example it is also beyond doubt that '...in biology the effect of one virus on one bacterium cannot be put into the forms of mathematics"! (FEYNMANN 1965.) The physicist with a Nobel-prize has a clear conception of this, and also that, a detailed description of the complicated situations needs help from the field of mathematics, but only if

we suppose to know the "fundamental laws of the game". FEYNMANN himself put it down as well "... so as toexpound and put into words the fundamental laws of the game, and for these laws mathematics are not needed". I do not intend to go into details here concerning FEYNMANN's conception, but a few thoughts taken from the work of SCHRÖDINGER titled "What's Life?" should be put down here "... the most important part of a living cell, the chromosome thread, can be called aperiodic crystal. In physics, up till now, only periodic crystals have been studied." Also "... Comparing the aperiodic crystals to the periodic ones they are very simple... The only difference between the structures is just like what it is between the common wall-paper on which the same pattern is repeated in a regular periodic system and between a master-piece made by a craftsman..., on the latter there is not any uninteresting repetition, but there is a detailed, meaningful pattern on it..." Then he continues his expositions as follows: "... the laws of physics have a natural tendency for switching over to inordinateness..., but life is based on the maintenance of the existent order." In a wider sense it means that living organisms by no means can be regarded as mechanical creatures, machineries, since "... they feed on negative entropy". Let me add something to it. When in a man-made mechanical structure only one element breaks down there is a fair chance that the whole machine will cease to work. In case of creature this phenomenon — taking into consideration the countless "security measures" and the presence of paired organs, etc. — it is not likely to happen.

Let it be distinctly understood that it is not my purpose to build up an opposition between mathematicians and ecologists, neither what has been said up till now has been proven the theory. It cannot be. Neither it is my intention to prove the superiority of the science of ecology an further on I would call your attention to that even "in our interdisciplinary age" there are disciplinary limits. It is not only a question of ethics. In brief (pointing the dagger in our direction) ecologists should become more acquainted with the characteristics and methodical possibilities within the laws of mathematics and physics, and then there would be no need for them to "run to" mathematicians all the time, since a mathematician should not be expected to be a biologist as well. In our times one of the most important tasks of ecology and of coenology is to find a harmony with mathematics and physics, mainly in the field of interpretation. In this sense a closer analysis of the question of the linguistic and metalinguistic verbalism is completely neglected area. Even though, particular analysis could at last

single out the appropriate proportions of applications, etc. (of which will be dealt with in more detail later in the book). In both aspects we should study SCHRÖDINGER...

The ecology of man-created ideas

It is clear from what has been said that there is only a slight harmony between the notions of ecology and the applied methods which have resulted in innumerable disturbing conceptual superimposition, e.g. in ethology, in plant-geography, and even in psychology. All these trace us back to the inconsistencies committed in the defining of the science. They cast light upon the ad hoc character of definitions. From these facts here emerges one of ARISTOTLE's witty sayings "... a slight mistake at the beginning will be big at the end...".

Here I would like to pick out only a few notions, the interpretations of which are rather disturbing. The first one is nothing else but aggression which has already slightly been touched upon. Being faithful to the undisciplined style, let us take a few approximations. The only aim of which is to get you into the spirit of this problem. Let us read the opinion of a psychologist: "Aggression is the name of all the intentional actions, the motive of which — in overt or symbolic form — to cause damage to somebody, to hurt somebody, or to give pain to somebody." (RANSCHBURG 1987.) It would hence follow that aggression is a deliberate, political action. Let us make an excursion into the field of ethology, and let us turn right away to Konrad LORENZ (1985): "... although animals have a purpose with purpose-orientated and changeable behaviour, the purpose itself must not be identified with the teleological sense of behaviour — as it was thought out by purpose-orientated psychologists. The animal as a subjective creature has an aim simply to let the inherited movement come into full display." From this meditation upon instincts only the following conclusion can be deduced: an animal cannot be aggressive "deliberately"... Why, we do not know for certain what instinct is itself, but — latently — its interpretation gets nearer and nearer to the conception of automaton (e.g. BENEDEK 1987).

Now let us get down to botany, with a question at the very beginning: Can a plant be aggressive? Obviously not. Although from the word weed comes "weeder". What this mean it can be a man or vehicle who get rid of weeds, and already the word "weeder" is becoming and accepted term in literature

and in everyday language. This word most of the time implies a group of words: oppress, exploit, drive out, extinguish, kill off etc. (e.g. UJVÁROSI 1973). KING (1966), RADOSEVICH and HOLT (1984) have collected some very interesting "definitions" about the so-called Weeds. For instance: "A plant growing where it is not desired." (Terminology Committee of the Weed Science Society of America 1956.) "A plant that grows so luxuriantly or plentifully that it chokes out all other plants possess more valuable properties." (BRENCHLEY 1920.) It is highly antropomorphic!

KING (1966) has said: "Surely a weed must be more than just a plant which in a particular place and at a particular time arouses human dislike."

SALISBURY's (1961) opinion must be quoted: "When not engaged in their nefarious activities both may have admirable qualities; a thief may be an affectionate husband and father outside business hours, while an aggressive weed in one environment may be a delightful wildflower in another."

It seems to me that RADOSEVICH and HOLT (1984) have the right view: "Most of the characteristics -- I mean the weed -- are based on some perceived human value such as aggressiveness, harmfulness, or undersirability. In this respect, weeds appear to be somewhat like the criminal element in human society."

After all these I think that we can ask another question, too, namely: what are the scientific grounds proposing that animals and plants are aggressive? Well, the terminological crisis has been represented by CSÁNYI (1986) himself. He says: "In the case of examining animals behaviour it is inevitable to use the conceptual constructions formed on the level of human social behaviour, and that involuntarily renders an anthropomorphic character to this view. Not having a critical conceptual analysis, research on aggression can hardly advance." Why then was it necessary to transplant this notion with force into the field of the world of plants and animals? The sociologist, BOOKCHIN (1982), the prize combatant of social ecology asks this question also: "Why do term borrowed from human social hierarchies acquire such remarkable weight when plant-animal relations are described? Do certain insects 'enslave' others? Does one species 'exploit' another?" It would be a great pity to disregard these remarks and not to put up the question; to what extent is the hierarchy of the biological world similar to the hierarchy of human societies?

We will overshoot the target again if we do not start with the fundamental questions: what does hierarchy mean? Plenty of imperfect approximations have been caused by the use of this notion (or concept), both in the

ecology and ethology. ODUM (1971): "Community, populations, organism, organ, cell, and gene are widely used terms for several major biotic levels shown in hierarchial arrangement from large to small..." (p. 4). From Chapter 9: "A communication network, some form of dominance hierarchy, learning, and a balance between contradictory behaviors (...aggression versus passiveness...) are the necessary ingredients for social organization..." (p. 250). In this concept living creatures are provided with high quality social consciousness...

There are extremely large problems exist in the classification of the communities. Neither the so-called "gradient analyses" nor the Gleasonian and Clementian aspects have been able to give a useful answer: What does mean community, or some question from the book of WHITTAKER: "How are species populations distributed in relation to one another and communities along an environmental gradient?" or "How are the kinds of communities in an area related to patterns of more than one environmental gradient?", and so on... Perhaps something wrong with the questions(?) It seems that these questions first of all invite to classification, creating new hierarchies between plant species or between plants and their herbivores etc.

The notion hierarchy can create such problems as if "everything should be connected with everything else", but this is not possible for everything. The explanation of these principles will bring forth exciting moments later in the text.

The conceptual interpretation of food chain (with its content and scope marks) is continuously being related to aggression. In this sense the expression "enemy" (e.g. STEINMANN 1985) is very wrong in its usage, even if it used in a book intended primarly to be popular work. Nobody has ever succeeded in proving that the members of the food chain are enemies to one another. It is a pity that even at primary school it is taught that lions are enemies of antelopes. If that was a fact then would not be any antelopes which would then decrease the number of prey which would lead to the starvation of lions which further would lead to extinction... It is correct that every creature is a kind of murderous "surviving machine"? This idea is created by DAWKINS (1976) when he refers to MAYNARD-SMITH's (1972) game theory as a background. It is a theory of DAWKINS that: "one surviving machine to another surviving machine (unless it not at all related) is just a part of its natural environment just like that of a rock, or a river, or that of a piece of food which is there, and which can be used by the surviving machine". A "surviving machine" is competable with rocks and rivers

become obstacles. When rocks and rivers become obstacles then the machine has to fight the obstacle so as to survive, and likewise another machine can be an obstacle, but as it is a "surviving machine" it retaliates. The natural selection — according to DAWKINS — is favourable for machines which construct their own "surviving machines" so as to exploit their surroundings as much as it is possible. I wonder if these careless statements can be said about the author himself. Anyway, the crature-machine conception has already been outworn, and some of us continue to interpret the biotic-abiotic relations in another way.

One of the most highlited topics in the science of ecology is the competition, and the results — based on field-works — show very interesting aspects in connection with the relations between competition and evolution; "The evolution of populations in directions that reduce competition thus leads both toward niche difference and toward habitat difference and the scattering of population centers along environmental gradients. Selection toward a distinctive niche and a distinctive habitat preference will normally occur at the same time, for niche and habitat are closely related aspects of the species' total adaptation to environment. However, the consequences of selection lead primarily away from, not toward, formation of groups of species with parallel distributions." (WHITTAKER 1975.) But what are the further ecogenital attributes of this tendency? Let us see DARWIN's work in connection with animals: "...on the various laws of inheritance, we learn that characters often or even generally tend to become developed in the same sex, at the same age, and periodically at the same season of the year, in which they first appeared in the parents. But these laws, from unknown causes, are very liable to change. Hence the successive steps in the modification of a species might readily be transmitted in different ways; some of the steps being transmitted to one sex, and some both; some to the offspring at one age, and some at all ages. Not only the laws of inheritance extremely complex, but so are the causes which induce and govern variability. The variations thus caused are preserved and accumulated by sexual selection, which is in itself an extremely complex affair, depending, as it does, or ardour in love, courage, and the rivalry of the males, and on the powers of perception, taste, and will of the female." ("Sexual selection" 1871.)

Hundred years later Konrad LORENZ — in the year of 1974 — has written his famous book about the "Naturgeschichte der Aggression". He writes: "Die Frage nach dem Arterhaltungswert des Kämpfens hat bekanntlich

schon Darwin selbst gestellt und auch schon eine einleuchtende Antwort gegeben: Es ist für die Art, für die Zukunft, immer von Vorteil, wenn der stärkere von zwei Rivalen das Revier oder das umworbene Weibchen erringt. Wie so oft, ist diese Wahrheit von gestern zwar keine Unwahrheit, aber doch nur ein Spezialfall von heute, und die Ökologen haben in jüngerer Zeit eine noch viel wesentlichere arterhaltende Leistung der Aggression nachgewiesen. Ökologie kommt von griechisch oikos, das Haus, und ist die Lehre von den vielfältigen Wechselbeziehungen, die zwischen dem Organismus und seinem natürlichen Lebensraum, seinem 'Zu-Hause', bestehen, zu dem natürlich auch alle anderen, ebenfalls dort lebenden Tiere und Pflanzen zu rechnen sind. Wenn nicht etwa die Sonder-Interessen einer sozialen Organisation ein enges Zusammenleben fordern, ist es auch leicht einsehbaren Gründen am günstigsten, die Einzelwesen einer Tierart möglichst gleichmässig über den auszunutzenden Lebensraum zu verteilen" (p. 37, 1988).

Recently it has become clear that in some conceptual elements both DARWIN and LORENZ were a step further than the majority of the ecologists of our century...

Striving for security

The situation is neither bright in the field of the interpretation of "behaviour". To quote from the "Ecological Encyclopaedia" (Hungarian edition): "In broader terms behaviour is nothing else than a reaction from human beings and animals, and these reactions can be observed externally." Is not there any difference between the intentional behaviour of human beings and the so-called instinctive, or acquired behaviour of animals?

We should give a more thorough consideration to whether the conception of behaviour can be applied to the interpretation in the outward manifestations of the beings which in present day belief have no intentions. It is interesting, but it cannot be accidental that besides the conception of behaviour there cropped up the conception of instincts, the question of mind, and from here the expression of ecological behaviour is not even a step away. It is apparent that conception chains follows each other just as in the law of gravity the planets do. When the concept and a scope of a concept seems to come to an end, on that point another concept joins it, and it is apparent that a conception cannot be interpreted without another, and could not exist without the other. This is especially true for the science

of ecology. Is there perhaps an interrelation between the conception network of varied sciences? I mean between psychology and ecology, between ethology and ecology. We can feel again the science of ecology "does not have any bounds". I wonder whether this feeling is completely "illegal". Why it is only a feeling? One thing is certain, we did not have a history — I mean a thorough history -- of ecology till now. We had very few imagination about the origin of the ecological thinking in human evolution and in philosophical and gnosiological thinking, and maybe because of its strong sensorial characteristic.

I wonder whether we can take it for granted or even avoid the ever-compelling conceptual approach that may exist an ecological behaviour, which concerns every organisms, and is realized in genetic mechanisms, also realized in acquired mechanisms, which would mean the pledge of successful adaptation. It is my belief that there exists such an ecogenetic behaviour, and one of my main tasks is to put it to proof. At first sight the involved horizon may seem too wide. I am completely aware to this, but still I would like to accentuate on it. We cannot go in for general ecology with complete success, but in the case of single organisms, in the case of populations of different species, and also in the case of biocoenoses it would render a possible method of approach. For the time being let us call this general characteristic "striving for safety". The conception of striving to be treated with reserve, since it can be used only if we talk about mammalia and the primates.

One thing is beyond doubt; the basis for the survival of the creatures depends on their propagation and caring for their offsprings which should take place in the best possible, secural conditions. DARWIN says: "The female often differs from the male in having organs for the nourishment or protection of the young, as the mammary glands of mammals, and the abdominal sacks of the marsupials. The male, also, in some few cases differs from the female in possessing analogous organs, as the receptacles for the ova possessed by the males of certain fishes, and those temporarily developed in certain male frogs. Female bees nave a special apparatus for collecting and carrying pollen, and their ovipositor is modified into a sting for the defence of their larvae and the community. In the females of many insects the ovipositor is modified in the most complex manner for the safe placing of the eggs." ("Sexual Selection", Princeton University Press, p. 254.) There is no oikos without security. Likewise there are no such species of plants or animals, neither is there a community in which the ecology of

security would not work. If it broke down it would result in the extinction of the organisms. This we will be able to see through the examples.

The striving for security can be regarded partially as an ecogenetic automation. In this case such life-moments, mainly ecophysiological attributes are involved which can be found in every creature as inherent, inner characteristics, e.g. at birth these life-moments start functioning "without" any external stimuli. We can put it into words as follows: inner, which means in this concept "built-in" moments of life, arise form the essence of living organisms, from the so-called ecogenetical principle. Although these life-moments do not take place without the environment, since the environment is always there, but there are situations in which part of the environment is irrelevant or passive for the organism in question. Swarmed with examples, we have to presume that the phenomena which we call ecogenetical principle and ecogenetical automation are universal in the living world, and they are of vital importance because they guarantee the ecophysiological basis of the security. Striving for security has got an enormously rich kind of attributes: adaptations, life-strategies, different sorts of behaviour, security chains, etc. belong to it, about which it will written in detail, accompanied by a wide range of examples, in the following articles.

I think it has already become clear by raising these problems that there is a need of reform in the field of conceptions. Very often the terms are not appropriately used, and they can darken the very reality, since the anthropomorphic phenomena may pass over rather easily to the different areas of zoology, ethology and botany. It can be a partial explanation to the fact that mainly ethology — in spite of the abundance in examples — rather easily skips over the fact that coexistence, built on security, can be much more advantageous for organisms and for their communities than the perpetual application of struggle and of aggression. The latter, regrettably, is one of the most specific characteristics of Homo sapiens.

Likewise it is a very interesting phenomenon that phytocoenology and plant-geography with its association-aspect and with its "sociological" approach avoided this trap (e.g. the works of SOÓ and BORHIDI). The interpretation of association involves a plant-association insuring security for its beings, for its single organisms and for its populations. To be more precise: in an association there live populations which co-operate with one another, they insure safe life-conditions for one another. This way they build up characteristic safety chains and networks etc.... We must stress that neither competitions nor food-chains are "excluded" from what has been

said, but we need more exact criticism against the superficial interpretation of these notions. Already niche-studies themselves draw attention to the fact that plant species do not play the game of ousting out the others, but they try to have a share (subject to their capabilities) in the given resource stock, to the most optimal degree. The momentums are realized in an actually existing phytocoenological operation (e.g. FEKETE et al., niche-studies since 1976).

It is becoming more and more urgent to interpret the conception "eco-genetics" appropriately. "Eco-genetics" is a term used also in the so-called vegetation-dynamics, primarily it deals with the "...niche species evolution...", respectively it marks the vegetation development in an area which is directed by natural ecological factors. It is a great pity that neither ecological nor genetic encyclopaedias include a usable definition of eco-genetics. CZEIZEL (1987) gives a few lines of explanation of it in his book in chapter "Human Eco-Genetics". As his explanation reads: eco-genetics refers to personal sensitivity, respectively, to the genetic element of it, as the resultant of the interactions of surrounding-organism.

In reality vegetation development, or the development either of an animal or of a plant, ontogenesis, too, can be interpreted and considered as the resultant if the interactions between genom (the total genetic material) and surroundings. It is the same resultant we can find a reference to in the definition of eco-genesis. In our time the so-called ecological genetics, is a fact, it is a special interface, but its topics are far from being uniform. Its perspectives are almost boundless, since we know very well that the extent of genetic determination can be influenced largely by the environmental effects which most of the time, unfortunately, are damages. These effects are manifest in developmental deformities, in blocked developmental stages etc. On these points already physicians are radically concerned: the effective collaboration between ecologists and physicians cannot be procrastinated any longer. Concerning to the whole scope of biology, embryological research can be exceptionally important, since deformities cannot be realized and detected early enough. There are several examples in developmental importance of lower plants that have a "deformatio-bioindicational" importance. These are the so-called "environmentally induced modifications", reflecting to the life-strategies of lichens (WEBER 1962, 1967, 1977, SEAWARD 1976, KISS 1985).

We have touched upon ontogenesis which simply attracts the conception of morphogenesis. Morphogenesis includes the series of morphological changes

of praembryonic stage, just like it includes the embryonic and postembryonic stages, and in the process of this happening internal and external, need security in organismic and ecological sense. Here we are at ecology again...

Organismic protection can be rendered by the womb, but persules and sepals can serve the same purpose too.

We know very little in botany about the structures which insure the development of offsprings. Likewise it is the same with the pollination process operation of organs, with the genetic background, as it is with the pollinational process and their interspecific ecological aspect. That is why I layed greater stress on flower-biological examples than it was ever done before. Ecology of security has got considerable organismic proofs. Having a thorough knowledge of flower-biology, will make us able to understand and get to know plant-animal relations better (e.g. KUGLER 1979, KRATOCHWIL 1983, 1984).

Morphogenesis makes it necessary to verify the results of research which examine only structures (e.g. patterns, layering etc.). Here, primarily, I think of verification of ecophysiological, cytological, morphological and taxonomical aspects. Anyway, we can take any field of biology, the test of models and of theories, the proving of their justness in practice, and the possibility of realization of justness lag behind the desired extent. It is to be remarked that this situation is partly due to the extremely complicated interaction-mechanisms that can be found between the living organisms and their communities. It happens in ever so many cases that the questions made up according to the demands of sciences would call forth not an adequate reaction from the organisms. In other words: a well-sounding raising of a problem, or just a question, can miss the point, researchers — not being aware of it — enter into other fields of reality, so in many cases we just fire round phenomena blindfolded.

The danger of the latter exists mainly when we disregard or try to disregard the individual and concrete elements of the process of cognition, disregarding the direct sensorial contact with the living organisms during the examinations, and we immediately want to get at general or abstract levels. We can obtain a posteriori (KANT 1787) knowledge with individual and concrete momentums only, then can we switch over to priority fields. As KANT's theory goes: "... even if all our knowledge begins with experience, not all of them originate from experience". Epistemology has been neglected by specialized branches of sciences to such an extent that in many cases we can see a sarcastic smile on POPPER's face as he announces: "Among the real

dangers to the progress of science is not the likelihood of its being completed, but such things as lack of imagination ... or a misplaced faith in formalization and precision ... or authoritarianism in one or another of its many forms." Or: "The history of science, like the history of all human ideas, is a history of irresponsible dreams, of obstinacy, and or error" (pp. 8, 9). We have to accept that the announcement — concerning a certain methodical situation -- proved to be true, within ecology. However the situation is far from being hopeless, since the birth of life-strategy conception and its widespread application in plant ecology, the conception of "permaculture" (1988 by Bill MOLLISON), will probably render wider possibilities for the interpretations, and at the same time, it will render wider feasibilities to the elaboration of the scientific background of an applied ecology.

The ecology of security is not intended to be a theory is forced upon organisms and on their communities, and it does not want to be something which is foreign to them either. The idea of the cology of security was brought into the world as a result of studying the life-cycles of plants, their life-strategies, the behaviour of animals, and last, but of course not at least, the studying of the evolution of man. Naturally we used a rich collection of examples taken from literature of relating sciences. Its conception have been formed primarily, as result of the inspiring effects of ecology, of ethology, of sociology, and of psychology, but genetics have left their essential marks there too.

I refer again to one of my aims: to analyse the relationship between Homo sapiens and nature. The time-scope of the examined material ranges from the palaeolithic age to our present day. My aim can, of course, be reached by an appropriate concentration on essentials, and by using adequate literary and proven material. The capability of elaborating a subject is limited. In the theme under discussion, there is not a known synthesis giving a new conception. The lack of which can be felt not only in scientific life but in everyday life as well. The conceptual failure in the protection of nature can be attributed to the false interpretation of the relation between men and nature.

Our age is the age of unscientific phrases (e.g. the protection of nature is a "self-defence"), it reflects a typically egoistic conception, and makes a diversion from nature itself.

Even though we do not know the origin of "ecological thought". We still have to unfold their germs from the ancient epics, e.g. from Sumerian,

Chinese, Hindi and European natural philosophies. Even this cannot do without the studying of the classicists of philosophy, psychology and of ethology. All these should be the task of a detailed history written on ecology. We should realize that each line, each sentence, opinion and conception bear the mental structure, and also bear the motivation of the person who created that particular work. In the formation of ecology, in our era, this phenomenon has got an exceptionally great importance. Even the pictographs give evidence of a specific attitude to nature.

I do not want to accentuate here that in my work a new conception was created, since most of the time in biology it is nature and not conceptions which triumphs. I tried simply to examine more thoroughly the aspect of a living world, which is a seemingly universal, and furthermore I tried to build my theory on examples.

Being faithful to the deviation from traditions, I would like to finish this introducing contemplation with the words of a commander, which will draw attention to the curious duality of human nature: "People look for hiding-places in villages, on beaches, in mountains. You yourself have longed for a hiding place. What an obtuseness! You can hide whenever you want, you can withdraw into yourself. Since you can withdraw into no better place which would be more serene, and less distrubed than your soul, especially if your inner world is such that after an inlook, it would fill you with complete peace. Peacefulness is nothing else but the harmony of soul. You should never, under any circumstances, deprive yourself of an insight the soul, and be revived by it" (Marcus Aurelius).

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NEOMEZIA VOTSCH EMEND. BORHIDI (THEOPHRASTACEAE), UN GÉNERO ENDÉMICO OLVIDADO DE LA FLORA DE CUBA

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Neomezia Votsch had been described for locating correctly the endemic Deherainia cubensis (Radklk.) Mez. The description (1904) was based mainly on anatomical features. Since that time the article has not been cited by any author dealing with Caribbean Theophrastaceae. In this paper the author re-evaluates the original description and amplifies it with recently detected new morphological features observed and studied on fresh flowering materials obviously not considered or observed by former authors. The new amplified set of the characters permits the author to confirm the validity of the forgotten generic name: Neomezia with a new description of the genus and a new combination: Neomezia cubensis (Radlk.) Votsch ssp. oligospinosa (Lepper) Borhidi.

Introducción

Al pié sombreado y en las fisuras húmedas de los paredones calizos de los mogotes de Cuba Occidental un arbustico de tallo rastrero con hojas coriáceas de margen espinoso fue colectado por Ch. WRIGHT y herborizado bajo el número 2916. GRISEBACH (1866: 163) indentificó esta planta como Theophrasta americana Sw., -- miembro de la familia Myrsinaceae — aunque con duda, mencionando las flores laterales solitarias y no racemosas, como caracteres diferentes de la descripción de MIQUEL. En 1890 RADELKOFER revisó el material y encontró que la planta cubana no es idéntica ni con la Theophrasta americana L., -- especie típica del género — ni con la Th. jussieui Lindl. (Th. americana Sw. non L.), ambas endémicas en Española. Pues, el describió la planta cubana como una especie nueva del género Theophrasta bajo el nombre Th. cubensis Radlk. En 1901 MEZ, especialista alemán de Myrsinaceae y Theophrastaceae, revisó las especies antillanas de la nueva familia, separó la planta cubana del género Theophrasta y la colocó al género mexicano Deherainia creando una nueva combinación: Deherainia cubensis (Radlk.) Mez. Para esta decisión MEZ tomó la forma y posición de los

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estaminódios, como criterio. El mismo concepto fue seguido por él en el tomo monográfico de la familia en Das Pflanzenreich IV.236a (1903). Peró el mismo MEZ admitió en esta obra (1903:3 y 7), que en la clasificación de este grupo quizás no se había encontrado la solución mas natural.

Por esta razón MEZ dió la tarea a VOTSCH, que confeccionara un estudio profundo sobre los caracteres anatómicos y la distribución de ellos dentro de la familia, para determinar su importancia taxonómica. VOTSCH en su trabajo (1904) estudiaba la anatomía foliar e caulinar de no menos de 70 especies de la familia Theophrastaceae. Subdividió la familia en dos subfamilias, dió una descripción anatómica de cada especie y una clave analítica para determinarlas en base de criterios anatómicos. En el caso de la *Deherainia cubensis* llegó a la consclusión, que esta planta tiene una combinación especial de los caracteres uniendo los anatómicos del género *Theophrasta* con los morfológicos florales del género *Deherainia*. VOTSCH qualificó estos criterios como suficientes para crear un género separado para la planta cubana nominandolo *Neomezia* en el honor de su maestro Carlos MEZ, profesor de la Universidad de Halle y creó una nueva combinación: *Neomezia cubensis* (Radlk.) Votsch.

Es un fenómeno inexplicable, que este trabajo y la descripción completamente correcta y legítima del género *Neomezia*, registrado también en el Index Kewensis, se ha quedado negligado por todos los autores que posteriormente estudiaban este grupo. URBAN no menciona este dato importante en ninguna de sus publicaciones innumerables, ni confirmandolo, ni rechazandolo. No aparece el nombre genérico *Neomezia* en la sinonimía de la Flora de Cuba de ALAIN (vol. IV. 1957: 114), ni en el articulo monográfico del especialista alemán, L. LEPPER (1982) tampoco. Este ultimo autor encontró una variabilidad infraespecifica notable entre las poblaciones que crecen sobre caliza y serpentinita respectivamente. En base de caracteres morfológicos cuantitativos y de anatomía foliar el separó dos subespecies: la típica ssp. *cubensis* de los mogotes, y la ssp. *oligospinosa* Lepper, endémica de la serpentinita de Cajalbana. LEPPER en su trabajo (1982) presenta estudios anatómicos foliares bastante profundos para apoyar su decisión taxonómica, tanto mas, que la descripción de la nueva subespecie esta basada en un ejemplar estéril. A pesar de esto, LEPPER no se refiere al trabajo fundamental de VOTSCH, obviamente desconocido por él.

Resultados

El autor que ya trabajó con anterioridad en otro género de la familia Theophrastaceae (BORHIDI & MUÑIZ, 1978), revisó toda la literature correspondiente a la cuestión de la taxonomía del complejo Theophrasta — Deherainia — Neomezia, para confrontar los argumentos de todos los autores anteriores que trabajaban en cada de estos géneros. Encontró que la descripción genérica de Deherainia y el diagnosis de la Deherainia cubensis contienen ciertas discrepancias e incertidumbres. Para poner algunos detalles cuestionados en claro parecía necesario hacer una revisión morfológica de la planta cubana. El autor logró a colectar materiales frescos en plena floración, que le permitió observar nuevas caracteristicas florales de importancia taxonómica, y también rectificar la descripción en algunos detalles que no fueron bien observables en material seco estudiado por los autores anteriores.

En esta forma se ha observado, que Neomezia no tiene corola campanulada, como Deherainia, sino urceolada, con un tubo de corola abruptamente contraido en el ápice. El tubo de la corola en la Neomezia esta connada hasta su 3/4-4/5 de su longitud, y no hasta la mitad que en la Deherainia. Los estaminodios de Neomezia aunque morfológicamente son parecidos a los de Deherainia, peró no estan situados entre los lóbulos de la corola en el ápice del tubo, sino en la mitad del tubo. Realmente los estaminodios de Neomezia estan completamente adnatos al parte superior del tubo de la corola y connados entre sí también formando un anillo carnoso, arcuado por debajo. Esta formación de los estaminodios es muy parecida a la que encontramos en la flor de la Clavija longifolia (Jacq.)Mez de Trinidad. Otros caracteres diferentes son, que los estambres de Deherainia no tienen un apendice caudiforme apical, lo que tiene Neomezia, y las anteras de Deherainia son elipticas, redondeadas en la base, mientras Neomezia tiene anteras aflechadas, de base aguda.

El caracter mas importante de Neomezia es probablemente el fenómeno de **seudosinandria**. Esta significa, que las anteras de Neomezia, aunque no son connadas, peró se ajustan completamente formando una cúpula sobre el ovario defendiendo el estigma. La cúpula de las anteras se termina arriba en los 5 apendices alargados hialinos de los estambres, que parecen ser 5 estilos. El fenómeno de la seuodinandria no aparece ni en Theophrasta ni en Deherainia, sino en el género Clavija, donde a veces las anteras no son connadas o aglutinadas, sino solamente coordenadas en un disco.

Table 1

Caracteres comunes y diferenciales de los géneros Deherainia, Neomezia y Theophrasta

Caracteres	Deherainia (Mexico, 2 especies)	Neomezia (O-Cuba, 1 especie)	Theophrasta (Española, 2 especies)
Estatura:	arbusto erguido	**arbusto postrado**	arbusto erguido
Tallo:	inerme	**espinoso**	**espinoso**
Hojas:			
margen:	entero	**espinoso**	**espinoso**
venación:	no reticulada	**reticulada**	**reticulada**
vasos en nervio medio:	unilacunar	**tri-a multilacunar**	**tri-a multilacunar**
cristales en epidermis:	presentes	**ausentes**	**ausentes**
Flores:	**laterales**	**laterales**	inflorescencia terminal
Corola:	campanulada	**urceolada**	campanulada
Anteras:			
forma:	aovada	**aflechada**	aovada
ápice:	truncado	**caudado**	**caudado**
base:	redondeada	**atenuada**	redondeada
situación:	libres	**coalitas**	libres
Estaminodios:			
forma:	**glanduliformes**	**glanduliformes**	mas grandes
posición en la corola:	ápice del tubo	**mitad del tubo**	base del tubo
Estigma:	discoidal	**globosa**	**globosa**
Seudosinandria:	ausente	**presente**	ausente

Para ilustrar la independencia taxonómica del género Neomezia y sus relaciones a los géneros Theophrasta y Deherainia, se presenta los caracteres comunes y diferenciales de los tres géneros discutidos en la tabla 1.

Por esta razón consideramos, que Neomezia no es un taxon intermediario entre Theophrasta y Deherainia, que une algunos elementos de ambos géneros en combinación especial, sino que representa una fase evolutiva importante de la familia Theophrastaceae, que tiene relaciones notables con el género continental suramericano Clavija también, y que por este debe considerarse como el género mas especializado del Norte del Caribe.

La descripción nueva del género Neomezia es lo siguiente:

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Frutices humiles, prostrati, ramuli apicem versus ferrugineo- vel brunneo-tomentosi vel villosi, sparse spinosuli, spinis stipularibus patentibus, basi villosis, superne glabribus et nitidis, 3-5 mm longis suffulti. Folia breviter stipitata, coriacea, margine incrassata et irregulariter spinoso-dentata, nervo medio supra impresso, subtus carinato, fasciculis

pluribus percurso, nervi laterales ditantes, utrinque prominuli, ante marginem arcuato conjuncti et dense reticulati, in spinas marginales non vel indirecte excurrentes, reticulo a fasciculis sclerenchymaticis utrinque valde prominentibus et dense dispositis obtecto. Lamina supra sparse lepidota, nitida, subtus densissime papillosa et opaca, transverse secta fibris subepidermalibus inferioribus in seriem duplicatam alteram epidermi accumbentem, alteram ab illa remote ordinatis in fasciculos magnos collectis, fibris spicularibus rarissimis, crystallis deficientibus.

Flores solitarii vel bini, laterales, e caule lignoso fasciculatim abeuntes, pedunculis tomentosis Sepala 5, imbricata, ovata vel oblongo-ovata, apice rotundata vel rariter acutiuscula, basi breviter connata, margine membranacea et brevissime fimbriolato-pilosa. Corolla urceolata, tubo globoso, superne incrassato-carnoso, usque at 3/4-4/5 longitudinis connato, apice abrupte contracto, viridi, lobi 5, imbricati, late triangulari-ovati, tubo 3-4-plo breviores, sub anthesin erecti, aurantiaco-rosei. Staminodia late trinagularia, minuta, petalorum tubi medio inserta, cum petalis perfecte connata, arcuatim inter sese conjuncta, annulum crasse carnosum circa partem superiorem tubi corollini formantia. Stamina 5, in tubo corollino inserta, corollae basi affixa, filamentis infime dilatatis in anillum brevissimum carnosum cum petalis connatum coalitis. Antherae oblongo-sagittiformes, extrorsae, grisaceo-nigrae, supra basin dorsifixae, basi attenuatae, apice longe caudato-appendiculatae, liberae sed arcte sibi stricte accumbentes et desuper visae cupulam ovarium styloque perfecte obtingentem efformantes, ante anthesim extrorse dehiscentes. Ovarium ovoideum, glabrum, in stylum longiorem contractum, stigmate globoso. Fructus globosus, usque ad 3-4 cm in diametro, brunneo-pubescens, stylo permanente. Semina obovata, leviter triangularia, 1-1,2 cm longa et 6-8 mm lata, endocarpio corneo.

Typus: **Neomezia cubensis** (Radlk.)Votsch in Bot. Jahrb. 33: 541. 1904.
Basionym: Theophrasta cubensis Radlk. in Sitz.ber. Bayr. Acad. 20: 188. 1890.
Syn.: Theophrasta americana Griseb. Cat. Pl. Cub. 1866: 163. non L. nec Sw. — Deherainia cubensis (Radlk.)Mez in Symb. Ant. 2: 437. 1901.
Holotypus: Ch. WRIGHT n. 567.=2916. GOET.
ssp. cubensis, in saxosis calcareis, Cuba: PR, Hab., Mat.
ssp. **oligospinosa** (Lepper)Borhidi **comb. nova.**
Basionym: Deherainia cubensis (Radlk.)Mez ssp. oligospinosa[1] Lepper, in Rev. Jard. Bot. Nac. Cuba, 3: 67. 1982.
Holotypus: ACUÑA 19412 HAC; Cuba, Pinar del Rio, Loma Pelada de Cajalbana, la Palma, leg.: ACUÑA, MAZA & MORRELL, 1949, in fruticetis serpentinosis.

[1]El nombre "oligospinosa" Lepper linguisticamente no es correcto siendo una palabra hibrida compuesta de elementos griego (oligos) y latino (spinosa). Correctamente el nombre subespecifico fuera "oligacantha" en griego o "paucispinosa" en latino.

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ESTUDIO TAXONÓMICO DEL GÉNERO RONDELETIA L. s.l. (RUBIACEAE) EN CUBA

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The author discusses critically the previous taxonomic treatments of the genus Rondeletia in broader sense. She evaluates the different taxonomic views and revises the morphological, anatomical and cytological characters used by former taxonomists. She analyses the composition of the characteristic features of the type of genus: Rondeletia americana L. and based on these studies determines the standard set of characters for genus. At the same time she excludes the taxa presenting a different set of characters and confirms the separation of the genera Acunaeanthus, Roigella both monotypic endemics of Cuba and the genus Suberanthus of Cuba and Hispaniola. The genus Rondeletia in narrower sense is a much more consice taxonomic units, although it still contains more than 130 species in the Antilles. For further subdivision of the genus Rondeletia ten sections are proposed. These are: I. Odoratae, II. Rigidae, III. Nipenses, IV. Calophyllae, V. Pedicellares, VI. Rondeletia, VII. Lindenianae, VIII. Leoninae, IX. Chamaebuxifoliae, X. Hypoleucae. Each of them is characterized by describing the main features, mentioning the type species and listing the species belonging to each section. The separation of the sections is tested with numerical methods. Analytical keys are also presented for Rondeletia and allied genera, for the sections of Rondeletia s. str. and also for the 64 Cuban species which are all endemics. The author gives detailed analyses of the separating features of the critical vicariant species, not described in a satisfactory way and therefore often misunderstood or neglected by non-specialists. A biogeographical review of the genus is also given with a special emphasis to the distribution patterns of the Cuban taxa.

Introducción

El género Rondeletia L. fué establecido por C. LINNEO (1753), en honor a G. RONDELET (1507—1566), médico y naturalista francés (BAILEY 1949), a partir de la especia Rondeletia americana L., de distribución bastante limitada, reportada de Jamaica y San Vicente.

El número de especies conocidas hasta el presente oscila alrededor de 140, con posibilidad que existan híbridos y formas extremas de una misma especie. ALAIN (1964) reportó para Cuba 60 especies.

Al iniciar nuestros estudios la sinonimia del género incluía los siguientes nombres: Petesia P. Br. (1756); Lighfootia Schreb. (1789);

Willdenovia J. F. Gmel (1791); Stevensia Poit. (1804); Willdenovia Stend. (1821); Arachnimorpha Desv. (1825); Arachnothryx Planch. (1849); Rogiera Planch. (1849).

Con la publicación del Tomo II del Index Kewensis (1895) se listan 85 especies del género Rondeletia; con posterioridad algunas pasaron a la sinonimia del mismo, por ejemplo R. chamaebuxifolia Griseb. (R. avenia Wr. ex Sauv.). Otras fueron separadas como tipos de diferentes géneros de la familia como Rondeletia verbenacea Griseb. = Ceratopyxis verbenacea (Griseb.) Hook. f. ex Hook., y Rondeletia phialanthoides Griseb. = Neomazea phialanthoides Krug et Urb.

En esa época no se conocía la distribución exacta del género porque se citaban especies de Asia Oriental, además de considerario representado en México, Centro América, N. de Sudamérica y Las Antillas.

Hasta aquí, observamos que dado el número de géneros que agrupa en su sinonimia es fácil comprender que el concepto genérico de Rondeletia fue ampliamente considerado por autores anteriores (BORHIDI et FERNÁNDEZ, 1981a y b) y tanto DE CANDOLLE (1830), PLANCHON (1849), BENTHAM y HOOKER (1873), HEMSLEY (1879), SCHUMANN (1897), STANDLEY (1918) y URBAN (1923—1928) no delimitaron con exactitud los caracteres de diagnosis para el género que limitarían la inclusión de otros grupos afines dentro del mismo; por tal sentido GRISEBACH (1866) describió tres especies que con posterioridad pasaron a former parte de otros tres géneros diferentes y STANDLEY (1918) al no reconocer una de las especies tratadas en sus secciones, la vuelve a describir ubicándola en otro género (BORHIDI et al., 1980).

Otro ejemplo elocuente lo constituye Stevensia Poit., endémico de La Española, que se distingue por su cáliz completamente cerrado en el botón abriéndose en dos lóbulos, como fue señalado por URBAN (1898) y debe excluirse de la sinonimía del género Rondeletia. Incluye ocho especies. Con anterioridad se expuso que PLANCHON (1849), dividió Rondeletia en tres géneros, segregando dos nuevos a partir de él, Rogiera y Arachnothryx, basándose fundamentalmente en la condición del orificio de la corola y el número de limbos florales (KIRKBRIDE, 1968). Con posterioridad diferentes autores los redujeron a la sinonimía de Rondeletia, hasta llegar a STANDLEY (1918), considerado buen especialista de las Rubiáceas continentales, quién en su revisión de las Rondeletias de Norte América mantuvo el concepto, creando un nuevo grupo para Rogiera y dividiendo Arachnothryx en dos nuevos grupos (BORHIDI, 1982), además estableció el límite genérico aceptado hasta la década del 60, en que STEYERMARK (1967) revalidó Arachnothryx en su

revisión de las especies suramericanas basado en los caracteres del fruto, semilla, testa, orificio de la corola, pelosidad del tubo de la corola, número de piezas florales, estípulas, pubescencia del hipantio y partes vegetativas aracnoideo o no.

Posteriormente, BORHIDI (1982) hace un estudio comparativo de las especies de Rondeletia de Norte América y encuentra diferencias genéricas esenciales entre las especies Antillanas y de América Central, coincidiendo con STEYERMARK (1967) en la revalidación de Arachnothryx, completando su trabajo; al mismo tiempo revalida el género Rogiera, en ambos casos amplía las descripciones anteriores, establece las nuevas combinaciones y señala los caracteres de diagnosis. Otra especie Centroamericana del género Rondeletia, ubicada por STANDLEY (1918) en un grupo monotípico aparte: Hondurenses, resultó pertenecer a otro género nuevo para la ciencia Javorkaea (BORHIDI y JÁRAI-KOMLÓDI, 1983), caracterizada por tener un tubo estipular largo, inflorescencia terminal cimoso racemosa, cáliz y corola 5-6-meros, zigomórficos, forma y estructura del polen diferentes de los patrones encontrados para Rondeletia.

Por otra parte podemos citar a DWYER (1980) quien hace una revisión de las especies de Panamá y acepta fundamentalmente el tratamiento seguido por KIRKBRIDE (1968) en su revisión de las Rondeletias panameñas, en éste sentido queremos destacar que la especie Rondeletia odorata Jacq. var. breviflora citada por KIRKBRIDE en su trabajo es un estado teratológico de la planta cultivada en el Jardín Botánico de Kew, por lo que la especie es cubana (FERNÁNDEZ et HERRERA, 1983). El resto de las especies citadas en el trabajo excepto R. panamensis, pertenecen a los géneros Rogiera y Arachnothryx. Al estudiar las especies cubanas agrupadas bajo éste género partimos de la base que no existía ningún trabajo previo que estudiara las Rondeletias de Cuba, aunque ALAIN (1964) compila las especies presentes en nuestra flora descritas por diferentes autores, hasta el momento de la edición de su obra. Las especies cubanas fueron estudiadas y descritas por diferentes científicos.

Fue preciso establecer los caracteres de diagnosis para el género, dado que incluía taxa muy disímiles unos de otros, con caracteres propios que hacían interpretar el concepto genérico en sentido amplio; y para una primera aproximación unificamos las especies según la estructura del ovario y del fruto, el tipo de polen, el tipo de placentación y el tipo de dehiscencia del fruto.

Partiendo del análisis de los rasgos anteriores y haciendo una revisión morfológica comparativa de las especies antillanas se inició este estudio y se comprobó que existían especies que no seguían el patrón general para los caracteres señalados y que habían llamado la atención de autores anteriores como especies singulares, como por ejemplo: Rondeletia correifolia y especies que incluian en su sinonimía otros géneros como Ferdinandea y Ferdinandusa.

La primera especie la describió A. RICHARD (1850) como Exostema neriifolium A. Rich., después GRISEBACH (1862) describe dos especies como miembros del género Ferdinandusa Pohl (Ferdinandea in Grisebach); F. stellata y F. brachycarpa. Posteriormente las tres especies fueron incluídas por Ch. WRIGHT, en SAUVALLE y WRIGHT (1873) en Rondeletia, K. SCHUMANN (1897) las reclasifica como miembros de la sección Gomphosia de Ferdinandusa, junto con F. elliptica Pohl. URBAN (1898) varía éste concepto considerándola mejor en Rondeletia. STANDLEY (1918) creó un nuevo grupo Stellatae dentro de Rondeletia para éste grupo de especies dudosas, pero al incluir en ella a R. subglabra indudablemente una Rondeletia típica, el grupo resultó ser heterogéneo y complejo.

Las evidencias anteriores ejemplifican la complejidad del grupo y avalan el por qué Rondeletia L. s.l. agrupa muchas especies; para su mejor delimitación y comprensión autores anteriores tales como GRISEBACH (1864) y STANDLEY (1918) lo subdividieron en grupo y secciones.

GRISEBACH (1864), destaca tres secciones, sección I: Petesia, sección II: Eurondeletia, sección III: Stevensia. Considero acertadas, para su tiempo, las secciones propuestas, aunque como es de esperar relacionó especies que hoy se ha comprobado pertenecer a otros grupos ejemplo Rondeletia laurifolia, incluída en la sección I donde no tuvo en cuenta el tipo R. stipularis (Petesia stipularis L., 1759), sin embargo unió otras con caracteres que permiten separarlas lo que hace que la sección II sea heterogénea pues no se basa en el tipo del género (R. americana) para ubical las especies; en la sección III no tiene en cuenta que Stevensia es endémico de Haití, como se ha confirmado y denominó su sección como tal. Como es de esperar no tuvo en cuenta las especies cubanas.

STANDLEY (1918), también hace una subdivisión del género Rondeletia en 15 grupos no denominados como secciones, donde incluye las especies cubanas descritas hasta esa fecha, los grupos son los siguientes:

Amoenae	Stellatae	Umbellulatae
Leucophyllae	Tinifoliae	Incanae
Laniflorae	Pedicellares	Pilosae
Calycosae	Odoratae	Hypoleucae
Hondurenses	Laurifoliae	Correifoliae

En la actualidad muchas de esos grupos no corresponden al género Rondeletia pues la conforman especies que pertenecen a otros géneros. ALAIN (1964) no divide el género en secciones.

2. Materiales y métodos

El material botánico utilizado consistió en todos los casos, en ejemplares de herbario debidamente herborizados, colectados en el campo durante las expediciones o los depositados en diferentes herbarios nacionales y extranjeros. La colecta y mantenimiento de las colecciones se realizaron por los métodos convencionales que existen, para el cuidado y conservación de ejemplares herborizados (FOSBERG y SACHET, 1965). El género se dividió en tres grupos principales para su estudio: Grupo 1: "correifolia"; Grupo 2: "Rondeletia s.s." y Grupo 3: "Rondeletia s.l.".

2.1. Morfología general

Se revisaron y estudiaron 3897 ejemplares, representantes de las floras de Las Antillas y Panamá, que pertenecen a los herbarios HAC, HAJB, S, BM, K, US, NY, L, B, GOET, GH, A, U, MICH, HMV, F, BP, VBI, acorde a las siglas del Index Herbariorum.

El análisis de la morfología general (órganos vegetativos y reproductivos) de las muestras se basó en la observación macro y microscópica complementándose con el análisis y estudio de los materiales bibliográficos localizados.

Se evaluaron y estudiaron los caracteres que a continuación se relacionan: dimensiones, forma y textura de las hojas; nervadura foliar; número de pares de nervious laterales; características morfológicas de las estípulas; tipo de inflorescencia y largo del pedúnculo; número de piezas florales; forma y tamaño del cáliz y de los lóbulos; forma y tamaño de la corola y de los lóbulos; forma y situación de los estambres y anteras; forma y pelosidad del estilo; forma del ovario y del disco; tipo de placentación; forma de inserción de los óvulos; tipo, forma y tamaño del fruto; tipo y forma de dehiscencia del fruto; forma y tamaño de las semillas; tipo de indumento en todos los órganos evaluados; caracteristicas morfológicas del grano de polen; epidermis foliar.

En los casos necesarios se realizaron mediciones (10, cuando fue posible) a diferentes parámetros (largo y ancho de las hojas, largo del pecíolo, largo de los lóbulos del cáliz, entre otros) y se evaluaron caracteres cualitativos como algunos de los citados con anterioridad.

Los datos obtenidos se compararon con las descripciones originales para los distintos taxa y con la literatura disponible en la actualidad sobre éste género (como se señala anteriormente), en aras de esclarecer la problemática taxonómica existente en el mismo y llegar a definiciones que se exponen en capítulos posteriores.

Los ejemplares examinados se compararon con los tipos depositados en los diferentes herbarios. Se realizó la tipificación de los nuevos taxa, según el método clásico, acorde con las normas y principios contenidos en el Código Internacional de Nomenclatura Botánica.

Hasta el momento es evidente, que se siguió para el estudio taxonómico el método tradicional, pero no se obvió, en los casos necesarios, el utilizar métodos complementarios que ayudan y enriquecen los resultados taxonómicos.

2.2. Morfología del polen

La morfología del polen se analizó en 19 especies, correspondiéndose, al menos, con un representante de los grupos en que se dividió el género para su estudio y con algunos de los géneros de la tribu Rondeletieae.

Se tuvieron en cuenta los resultados obtenidos por Moncada, M., en sus investigaciones palinológicas sobre ésta familia.

Las muestras tomadas fueron acetolizadas según el método de ERDTMAN (1960, 1969). Se realizaron las mediciones correspondientes al diámetro de los granos de polen, determinándose forma y tamaño; se observaron detalles de la exina y tipo de apertura. En la descripción de la morfología del polen se utilizó la terminología propuesta por ERDTMAN (1963). Las muestras se examinaron en los microscopios ópticos Zeiss NV, Opton y Amplival. Las observaciones de microscopía electrónica de barrido y las microfotografías se hicieron el el microscopio electrónico Jeol 35, de las Universidades de Budapest y de Uppsala respectivamente. Las preparaciones permanentes están depositadas en las colecciones del Departamento de Botánica del Instituto de Botánica y Ecología, Vácrátót, Hungría y en la palinoteca del Herbario del Instituto de Ecología y Sistemática (HAC); réplicas de las preparaciones de polen y los originales de los negativos de las fotos tomadas al microscopio electrónico de barrido se encuentran depositadas en las colecciones del Departamento de Taxonomía de Plantas y Ecología de la Universidad de Budapest, Hungría.

2.3. Nervadura

Para estudiar el patrón de venación de la hoja, se utilizaron en todos, los casos muestras de ejemplares de herbario y se procedió a aplicar la técnica de diafanización de DIZCO (1973). Se analizaron 12 muestras y todas se sometieron a calentamiento previo con agua glicerinada, para hidratar la hoja. Posteriormente para obtener la transparencia de la muestra y poder teñirla, se introduce en una solución de hipoclorito de sodio al 50%; finalmente después de embebida la misma en hidrato de cloral al 5%, para eliminar opacidad, se procede al montaje permanente. Se realizaron algunas modificaciones en la temperature, tiempo de inclusión en los diferentes reactivos, así como en las concentraciones de las soluciones a utilizar, según MARTINEZ (com. pers.) producto de la diferencia en la textura de las hojas de éstos taxa y ser todas muestras herborizadas que inicialmente no respondían o demoraban el procedimiento normal que se aplicaba. La terminología utilizada en la clasificación es la propuesta por HICKEY (1973). Se utilizó para las observaciones un microscopio estereoscópico Olympus (aumento de 8x y 10x).

2.4. Epidermis foliar

A 12 muestras correspondientes a los tres grandes grupos en que se dividió el género para su estudio y evidenciando su complejidad taxonómica, se le aplicó la siguiente técnica, basada en la obtención de la cutícula a partir de un fragmento de un cm^2 de la región central de la hoja (incluyendo el margen). La porción tomada se calentó en una solución de 1:1 de peróxido de hidrógeno y ácido acético hasta que se separaron las cutículas por los bordes.

Después se procedió a lavar con agua fría; y se extrajo el mesófilo de la hoja con ayuda de un pincel, por último se montó en un porta objeto con solución de Ferrant y se realizó la preparación permanente. Se hizo el análisis de las superficies abaxial y adaxial de las hojas; para determinar las dimensiones de los estomas, se realizaron 10 mediciones en cada muestra del largo y ancho de las células oclusivas y de la apertura estomática. Las observaciones se realizaron en un microscopio óptico Lavobal. Además, las hojas se sometieron a estudio utilizando las técnicas de microscopía electrónica.

En los estudios anatómicos también se consideraron (cuando fue necesario) los resultados obtenidos por VALES (1982, 1983) al estudiar la anatomía de la madera de especies de interés, para el desarrollo de ésta investigación.
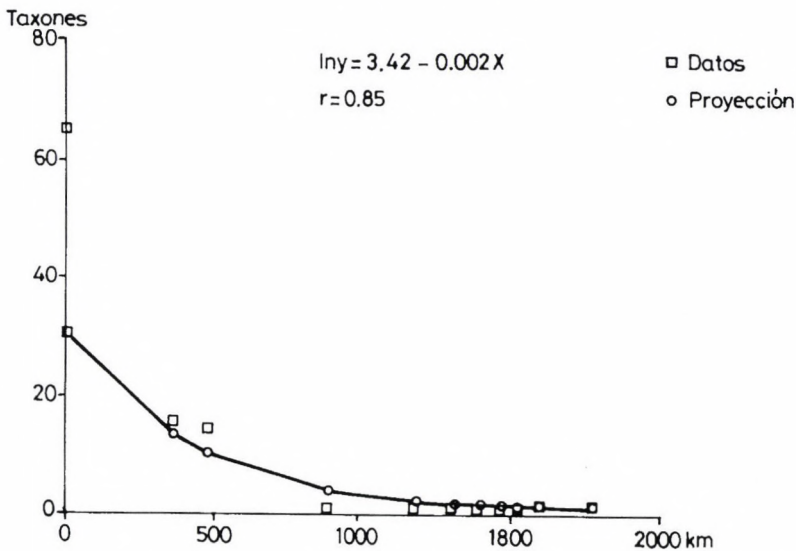
Fig. 1. Correlación entre el número de especies y la distancia a Cuba (Km)

2.5. Geográficos y fitogeográficos

Para determinar el área de distribución de los taxa, tomamos los datos de localidades de los ejemplares representados en los herbarios. De éstos datos y de nuestras observaciones de campo determinamos las caracteristicas ecológicas y corológicas mas notables de los grupos estudiados.

Se confeccionaron esquemas de distribución. Con los datos de distribución de las especies del género, se calculó la correlación logarítmica entre la distancia (Km) a Cuba de las tierras vecinas y el número de especies presente en cada una. Se trazó la curva de regresión correspondiente definida por lny = 3.42 - 0.0024 (Fig. 1), para ello se utilizó el lenguaje Super Cal-4.

Para los criterios fitogeográficos se tuvieron en cuenta las clasificaciones de SAMEK (1973) y BORHIDI y MUÑIZ (1986) y para definir las formaciones vegetales donde crecen las plantas representadas en Cuba, se siguió la clasificación de CAPOTE y BERAZAÍN (1984).

2.6. Métodos estadísticos

En los casos de especies que mostraban cierta variabilidad en su morfología externa, se realizó el estudio de los caracteres variables; los datos cuantitativos permitieron obtener la media de los valores y hallar el índice de relación (R) largo/ancho de las hojas (FERNÁNDEZ y HERRERA, 1983; FERNÁNDEZ y BORHIDI, 1984; FERNÁNDEZ y ECHEVARRÍA, 1988). Para el análisis de la variabilidad se aplicó la dócima de T (Student), para los índices largo y ancho de las hojas, largo del peciolo, largo de las estípulas, largo hasta la parte mas ancha de la hoja; para ello se utilizó el Paquete de Programas Estadístico MICROSTAT, 1985.

Los grupos estudiados se separaron, en sentido general, según los criterios de taxonomía clásicos y con el fín de corroborar ésta separación atendiendo al parecido, desde el punto de vista macro-morfológico se aplicó el coeficiente de similitud de Jaccard, contenido en el Programa CNCLAS, del IES-ACC. El índice de similitud (Sjs), se calculó sobre la base de 29 y 44 caracteres para comparar secciones y géneros respectivamente (tablas 1 a 4); éstos taxa

Tabla 1

Caracteres utilizados en el cálculo del coeficiente de similitud entre las secciones del género Rondeletia

1. Ramas cilíndricas pelosas
2. Estípulas pelosas pequeñas (0,5 mm-2 mm)
3. Estípulas triangulares subuladas o acuminadas
4. Peciolos antrorso pelosos, mayores de 8 mm
5. Hojas mayores de 2 cm
6. Apice agudo a obtuso
7. Base acorazonada
8. Base estrecha a obtuso-redondeada
9. Margen recurvo
10. Textura variable
11. Domacias ausentes
12. Inflorescencia axilar
13. Inflorescencia 1-3 floras
14. Inflorescencia cimosa
15. Pedúnculos 1-3 cm, pelosos
16. Pedicelos 1-5 mm, pelosos
17. Brácteas pequeñas
18. Pétalos y sépalos 5
19. Sépalos iguales
20. Sépalos entre 2-4 mm
21. Sépalos libres
22. Sépalos triangular-espatulados
23. Corola pequeña, hasta de 6 cm
24. Tubo de la corola retrorso-peloso
25. Pétalos glabros o glabrescentes
26. Semillas aladas o apendiculadas
27. Cápsula mediana, hasta 5 mm
28. Cápsula pelosa
29. Cápsula globosa

se consideraron Unidades Taxonómicas Operacionales (UTO) en cada análisis, respondiendo a la fórmula Sjs = (a/a+b+c)·100 (SOKAL y SNEATH, 1963). Con los coeficientes calculados se confeccionaron las matrices de afinidad y los dendrogramas correspondientes (Fig. 2).

Por otra parte, con los datos obtenidos, se aplicó un análisis de componentes principales, para lo cuál se usó el Paquete de Programas STAT-itcf; se utilizaron 27 caracteres para evaluar las secciones, confirmándose, cuáles incidían de forma mas aguda en la formación de los grupos que aparecían. Los gráficos representan los taxa con respecto a los tres primeros ejes (F1, F2 y F3) (Fig. 5) se confeccionó el dendrograma correspondiente (Fig. 6).

Tabla 2

Distribución de los caracteres utilizados en las diferentes secciones

Caracteres	Secciones									
	I	II	III	IV	V	VI	VII	VIII	IX	X
1.	X	X	X	X	X	·	X	X	X	X
2.	·	·	·	X	X	·	X	·	X	X
3.	X	·	·	X	X	·	·	X	X	·
4.	X	·	·	X	·	X	X	X	·	·
5.	X	X	X	X	X	X	·	X	X	·
6.	·	·	X	·	X	X	·	·	X	·
7.	X	X	·	·	·	X	X	·	X	·
8.	·	X	X	·	X	·	·	·	X	X
9.	·	X	X	·	X	·	·	·	·	X
10.	X	·	·	·	X	X	·	X	X	X
11.	·	·	X	·	X	·	X	·	X	X
12.	·	X	·	·	·	X	X	X	X	X
13.	·	·	X	·	·	·	X	·	X	X
14.	X	·	X	X	·	·	X	·	·	·
15.	·	·	X	·	X	X	X	X	·	·
16.	·	·	X	·	·	X	X	·	X	X
17.	·	·	X	·	X	X	X	X	X	X
18.	·	X	·	X	·	X	X	·	·	·
19.	X	X	X	X	X	X	X	X	X	·
20.	·	·	·	·	X	·	·	X	X	X
21.	X	X	·	X	X	X	X	X	·	·
22.	X	X	X	·	·	X	·	·	·	·
23.	·	·	·	X	·	X	·	·	X	X
24.	X	·	X	·	X	X	·	·	X	X
25.	·	X	X	·	X	·	·	·	·	X
26.	·	X	·	X	X	·	X	X	X	·
27.	X	·	X	X	·	X	X	X	·	·
28.	X	·	X	·	·	X	X	X	X	X
29.	X	·	X	·	X	X	X	X	X	X

Tabla 3

Caracteres utilizados en el cálculo del coeficiente de similitud entre géneros afines

1. Ramas cuadrangulares, pelosas
2. Estípulas grandes, mayor de 5 mm
3. Estípulas deltoideo-cuspidadas
4. Pecíolos nulos
5. Hojas mayores de 4.5 cm
6. Hojas glabras en ambas caras
7. Apice variable
8. Base obtusa a acorazonada o subacorazonada
9. Margen plano a subrevoluto
10. Hojas coriáceas
11. Domacias ausentes
12. Nervadura reticulada
13. Inflorescencia axilar
14. Inflorescencia uniflora o pauciflora
15. Inflorescencia cimosa
16. Inflorescencia glabra
17. Pedúnculos pelosos, mayores de 1 cm
18. Pedicelos pelosos, entre 1-5 mm
19. Brácteas grandes, foliosas
20. Sépalos y pétalos 5-6
21. Sépalos iguales
22. Sépalos mayores de 4 mm
23. Sépalos libres
24. Sépalos triangular-espatulados
25. Corola màyor de 1 cm
26. Tubo de la corola pubescente
27. Pétalos pelosos en ambas caras
28. Estilo glabro
29. Semillas angulosas
30. Polen subesferoidal o elíptico subesferoidal
31. Polen 4-5 colporado
32. Placenta con inserción central
33. Garganta de la corola glabra
34. Anillo faucial ausente
35. Cápsula mayor de 5 mm
36. Cápsula lenticelada
37. Cápsula piriforme
38. Superficie adaxial con paredes anticlinales hundidas
39. Superficie adaxial con paredes anticlinales elevadas
40. Superficie abaxial con paredes anticlinales hundidas
41. Superficie abaxial con paredes anticlinales elevadas
42. Pelos simples con estremos agudos
43. Longitud de los estomas, menor de 10 µm
44. Ancho de los estomas, menor de 10 µm
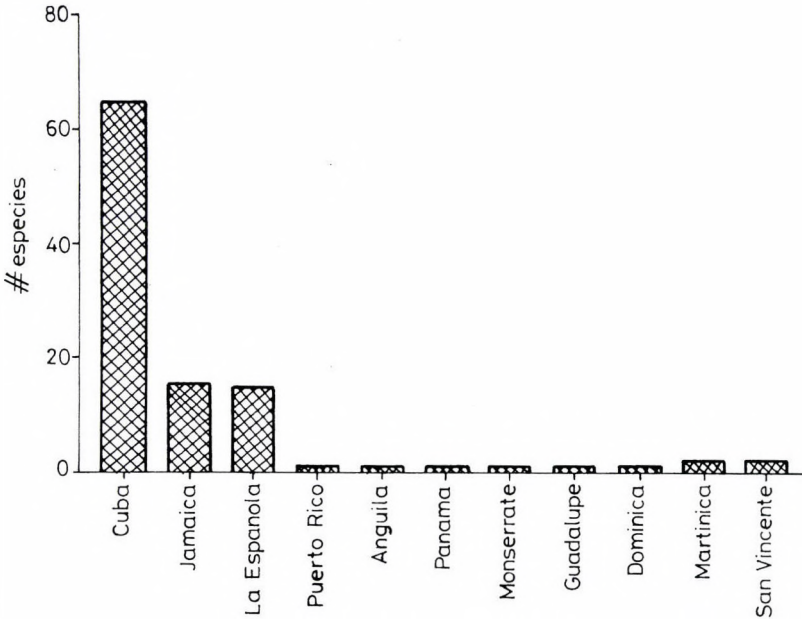
Fig. 2. Representación de la distribución de especies de Rondeletia en el área

3. Resultados y discusion

3.1. Morfología general

La evaluación taxonómica de las caracteristicas morfológicas es un instrumento fundamental del trabajo en sistemática; por lo cual dos tipos de evaluaciones se realizaron:

-- evaluación de la estabilidad y variabilidad de los caracteres y

-- evaluación de la importancia filogenética de los caracteres.

Después de analizar éstos aspectos y teniendo en cuenta que el género se concebía de forma amplia y que es polimorfo, se delimitaron los caracteres fundamentales para la separación de los taxa en tres grupos principales (como se señala en el capitulo 2); dos de ellos constituyeron nuevos reportes para la ciencia: Roigella BORHIDI et FERNÁNDEZ, 1981a (grupo correifolia) y Suberanthus BORHIDI et FERNÁNDEZ, 1981b (grupo Rondeletia s.l.); Rondeletia L. (grupo Rondeletia s.s.) se dividió en secciones, como sigue: Odoratae (I), Rigidae (II), Nipenses (III), Calophyllae (IV), Pedicellares (V), Rondeletia (VI), Lindenianae (VII), Leoninae (VIII), Chamaebuxifoliae (IX) e Hypoleucae (X).

Tabla 4

Distribución de los caracteres evaluados en el estudio de los géneros

Caracteres	Géneros		
	Rondeletia	Suberanthus	Roigella
1.	1	0	0
2.	0	0	1
3.	0	0	1
4.	0	0	1
5.	0	1	1
6.	0	0	1
7.	1	0	1
8.	0	0	1
9.	0	1	1
10.	0	1	1
11.	0	1	1
12.	0	1	1
13.	0	0	1
14.	0	0	1
15.	0	1	1
16.	0	1	0
17.	0	0	1
18.	0	0	1
19.	0	0	1
20.	0	0	1
21.	0	0	1
22.	0	0	1
23.	0	1	1
24.	0	1	1
25.	0	0	1
26.	0	0	1
27.	0	0	1
28.	0	1	0
29.	0	0	1
30.	0	1	1
31.	0	0	1
32.	1	0	1
33.	0	1	1
34.	0	0	1
35.	0	1	1
36.	0	1	0
37.	0	1	0
38.	0	1	0
39.	0	1	0
40.	0	1	0
41.	0	1	0
42.	0	1	0
43.	0	0	1
44.	0	0	1

En este capítulo se expondrán y discutirán los criterios que permitieron arribar a estos resultados. La definición de los caracteres de diagnosis para Rondeletia s.s., la revalidación de diferentes géneros a partir de su sinonimía (STEYERMARK, 1967; BORHIDI, 1982) y la descripción de otros nuevos para la ciencia (BORHIDI y FERNÁNDEZ, 1981a y b; BORHIDI y JÁRAI-KOMLÓDI, 1983), contribuyeron a delimitar taxonómicamente el mismo, lo que implicó la limitación de su distribución geográfica.

3.1.1. Hábito

Este es un carácter de gran valor a nivel genérico, pero la distribución de las formas de crecimiento en la familia indica tendencias definidas (VERDCOURT, 1958).

Las especies de Rondeletia son arbustos o pequeños arbolitos siempreverdes que varian entre 1 y 5 m de alto; al igual que los miembros de Roigella y Suberanthus cuyo porte es similar, alcanzando el primero hasta 3 m de altura y el segundo oscila entre 2-6 m, llegando algunos hasta 8 m de alto.

No existen en los grupos tratados representantes herbáceos; hábito que se considera derivado; por lo que en general los taxa estudiados presentan en éste aspecto caracteres primitivos tanto por el hábito como por la permanencia de sus hojas durante toda su vida.

Las ramas son cilíndricas o cuadrangulares en Rondeletia, generalmente angulosas en Roigella y la mayoría cilíndricas en Suberanthus.

3.1.2. Estípulas

A pesar de ser éste un carácter de valor diagnóstico para la familia, las mismas varían de un género a otro e incluso a nivel específico. Pueden ser interpeciolares, intrapeciolares o envainadoras.

En Rondeletia se aprecia esta variabilidad a nivel específico y en sentido general hemos establecido algunos patrones desde el punto de vista morfológico para ubicar las secciones. Estípulas triangulares a anchamente triangulares, cuspidadas, subuladas o acuminadas presentan las secciones I, IV, V, VIII y IX; deltoideas es característico en la sección III; largo cuspidadas exhibe la sección VII; triangulares se aprecia en VI; aovado-lanceoladas en II y la sección X muestra gran variación morfológica entre sus representantes, que no permite ubicarla dentro de un patrón específico.

El tamaño de las mismas también varía como sigue, mayores de 5 mm (Rigidae); entre 2,5 y 4,5 mm (Nipenses, Rondeletia y Leoninae) y las más

Tabla 5

Valores de los vectores propios de las variables en los dos análisis

Caracteres	Vectores propios					
	Ejes ACP-1			Ejes ACP-2		
	F1	F2	F3	F1	F2	F3
Ramas	.11	.26	.14	.24	.20	.07
L. peciolo	.05	.33	.07	.22	.23	.22
L. Hojas	.03	.26	.19	.17	.13	.33
F. apice	.25	.20	.13	.37	.09	.14
F. base	.01	.26	.23	.19	.28	.09
Margen	.01	.30	.26	.20	.34	.14
Posicion inflorescencia	.27	.19	.16	.37	.12	.17
Numero flores	.25	.21	.09	.13	.41	.08
Tipo de inflorescencia	.27	.07	.08	.31	.12	.16
L. pedunculo	.30	.08	.22	.24	.38	.06
L. pedicelo	.19	.01	.27	.20	.28	.16
Bracteas	.31	.05	.10	.25	.20	.20
Semejanza entre sepalos	.15	.25	.09	.01	.29	.25
Indumento tubo corola	.20	.32	.03	.38	.10	.10
Indumento petalos	.03	.33	.23	.23	.34	.07
L. Fruto	.20	.04	.34	.22	.03	.39
Indumento fruto	.05	.05	.37	.08	.04	.45
F. Fruto	.05	.00	.42	.01	.06	.47
Dimensiones estipulas	.17	.10	.15			
F. estipulas	.00	.15	.25			
Indumento hojas	.16	.18	.10			
Textura hojas	.19	.01	.02			
Numero sepalos y petalos	.25	.20	.05			
L. sepalos	.20	.10	.07			
Fusion entre sepalos	.24	.23	.04			
F. sepalos	.21	.06	.21			
L. corola	.24	.03	.09			

pequeñas oscilan entre 0,5 y 2 mm generalizadas en las secciones Calophyllae, Pedicellares, Lindenianae, Chamaebuxifoliae e Hypoleucae. Las de Odoratae son muy variables en su longitud.

Roigella tiene estípulas deltoideo-cuspidadas, entre 5 y 7 mm de largo. Suberanthus las presenta triangulares, coriáceas y pequeñas, entre 0,5 y 2 mm de largo. Desde el punto de vista filogenético, éste es un caracter primitivo para la familia, criterio que se comparte con los diferentes autores (clásicos y contemporáneos).

3.1.3. Hojas

El patrón que distingue a la familia Rubiaceae son sus hojas opuestas, simples, enteras y con estípulas, TAKHTAJAN (1980) señala que las hojas de

las plantas con flores, vivientes, primitivas son principalmente simples, enteras, con nerviación pinnada, coriáceas y glabras, lo que indica que los grupos tratados exhiben caracteres de primitivismo definidos, que comparten con otros derivados como lo es el ordenamiento de sus hojas opuestas o verticiladas. Estos caracteres en sentido general son válidos para Rondeletia y taxa afines; aunque pueden presentarse más de dos hojas por nudos, como en R. pedicellaris Wr. ex Sauv. y en Suberanthus spp. Todas son siempreverdes.

Rondeletia tiene hojas de textura variable, pueden ser coriáceas (Odoratae, Nipenses, Rigidae, Pedicellares, Secc. Rondeletia, Leoninae, Chamaebuxifoliae e Hypoleucae), subcoriáceas (Nipenses, Calophyllae, Lindenianae, Leoninae, Chamaebuxifoliae e Hypoleucae), cartáceas (Odoratae y Pedicellares), membranáceas (Secc. Rondeletia y R. microphylla); el margen se presenta de plano a revoluto, atravesando por estados intermedios. Algunos presentan el limbo de la hoja plegado o rugoso, ejemplo, algunas especies de Hypoleucae; otras especies tienen el haz brillante o lustroso como Calophyllae y Secc. Rondeletia.

Roigella presenta hojas coriáceas y el margen es plano a subrevoluto.

Las hojas de Suberanthus son coriáceas y generalmente opuestas o ternadas; el margen es plano a subrevoluto; al secar el ejemplar se torna oscuro (carmelita oscuro a negruzco), casi siempre son brillantes ó lustrosas en el haz. Algunas especies de Rondeletia (R. grandisepala Alain, R. odorata Jacq. ssp. odorata) así como Roigella correifolia (Griseb.) Borhidi et Fernández tienen hojas sésiles, mientras que el resto de las especies de Rondeletia y Suberanthus tienen peciolos de longitud variable, desde 0,5 mm hasta mayores de 8 mm.

Las formas de las hojas son muy variables en Rondeletia presentándose elípticas, obovadas, oblongas, lanceoladas, oblanceoladas, aovadas, ovales, orbiculares, cuneadas, mostrando todas las posibles combinaciones entre estos patrones principales.

STEBBINS (1974) apunta que las hojas de las angiospermas originales son elípticas, obovadas ó espatuladas, estrechando hacia la base; si tenemos esto en consideración se aprecia que éste taxon en cuanto a éste caracter combina caracteres primitivos y derivados, lo que se observa en todas las secciones excepto en Lindenianeae que en Cuba la representa un taxon con hojas obovadas.

El tamaño de las hojas también es variable, dado por la riqueza del género en cuanto al número de especies, de ahí que existan cuatro secciones (Rigidae, Calophyllae, Secc. Rondeletia y Leoninae) que se caracterizan por

tener hojas grandes, mayores de 4,5 cm como promedio, otras cuatro (*Nipenses*, *Pedicellares*, *Lindenianane* y *Chamaebuxifoliae*) presentan hojas entre 2 y 4 cm y la sección *Hypoleucae* muestra hojas menores de 2 cm. Se destaca *Odoratae*, con *R. odorata* en Cuba, como representante de la variabilidad foliar ya que incluye todos los rangos antes señalados (FERNÁNDEZ y HERRERA, 1983). Las formas de los ápices y bases también permiten enfatizar la variabilidad foliar de éste género. Las secciones *Rigidae*, *Rondeletia*, *Lindenianae* y *Chamaebuxifoliae* tienen hojas con ápices agudos a obtusos; los de las secciones *Nipenses*, *Calophyllae*, *Pedicellares* e *Hypoleucae* varían de obtuso-agudos a redondeados; *Leoninae* muestra ápices acuminados a agudos y *Odoratae* tiene formas cuspidadas a cortamente acuminadas ó redondeadas.

Rondeletia, *Lindenianae* y *Leoninae* tienen base cuneada o simplemente la hoja se va estrechando hacia el pecíolo, la base de *Odoratae* y *Calophyllae* es obtusa a acorazonada ó subacorazonada y bases estrechas a obtuso-redondeadas están presentes en *Rigidae*, *Nipenses*, *Pedicellares*, *Chamaebuxifoliae* e *Hypoleucae*.

Roigella tiene hojas grandes, que pueden alcanzar hasta 8 cm de largo y varían de ovales a oval-oblongas, con el ápice redondeado a obtuso, a veces apiculado y la base acorazonada o subacorazonada.

Hojas obovadas u oblongas son las formas que predominan en *Suberanthus*, son las mayores de los tres géneros y pueden llegar hasta 11 cm de largo. El ápice es redondeado a obtuso, a veces agudo; hacia la base se va estrechando el limbo foliar en forma cuneada ó aguda.

3.1.4. Indumento

Con excepción de *Suberanthus* que es glabro, las ramas de los géneros estudiados presentan un indumento que varía de pubérulo a hírtulo, pelosidad que en la mayoría de los casos se va perdiendo con la edad.

El indumento del peciolo es variable en *Rondeletia* incluyendo pelos antrorsos, retrorsos o hirtos; en *Suberanthus* es glabro excepto en *S. brachycarpus* (Griseb.) Borhidi et Fernández. Hojas glabras en ambas caras se aprecian en *Roigella* y en la sección *Calophyllae*; glabras o con pelos antrorsos en los nervios por el envés tienen *Rigidae* y *Lindenianae*; glabras o escabrosas en el haz con pelos antrorsos o estrigosos en los nervios por el envés en *Odoratae*, glabras o glabrescentes en el haz son las hojas de *Nipenses*, *Pedicellares*, Secc. *Rondeletia* y *Chamaebuxifoliae*. *Hypoleuceae* y *Leoninae* presentan un tipo de pelosidad variable, aunque en *Hypoleucae* generalmente son tomentosas en ambas caras y con pelos mayormente retrorsos.

Suberanthus tiene hojas mayormente glabras excepto S. brachycarpus que son mayormente pelosas o antrorso pelosas en los nervios por el envés.

Las estípulas en Rondeletia y Roigella son seríceas y en Suberanthus glabras.

Domacias o mechones de pelos en las axilas de los nervios por el envés están ausentes en Roigella y Suberanthus. En algunas especies de las secciones Odoratae, Calophyllae, Rondeletia y Leoninae están presentes así como en la sección Rigidae; en el resto de los taxa que conforman el género están ausentes.

3.1.5. Inflorescencia

En Rondeletia la posición de la inflorescencia es variable, puede ser terminal, axilar o ambas. La sección Odoratae tiene mayormente inflorescencias axilares a veces terminales; Nipenses, Calophyllae, Pedicellares e Hypoleucae las muestran terminales y axilares y Rigidae, Rondeletia, Lindenianae, Leoninae y Chamaebuxifoliae tienen inflorescencias axilares. El número de flores es también variable, de multifloras a paucifloras. Multifloras se observan en Calophyllae y Rondeletia, unifloras o paucifloras son las inflorescencias de Rigidae, Pedicellares y Leoninae. De 1-3 floras tienen las secc. Nipenses, Lindenianae, Chamaebuxifoliae e Hypoleucae; mostrando la secc. Odoratae un número variable de flores.

En cuanto al tipo la mayoría de las especies exhiben inflorescencias cimosas, lo que corresponde con el patrón típico para la familia en éste aspecto; TAKHTAJAN (1959, 1964) y STEBBINS (1974) señalan que éste tipo de inflorescencia es el mas primitivo y que la forma más primitiva de inflorescencia cimosa es probablemente una cima hojosa simple, terminal poco florida.

Las secciones de Rondeletia muestran diferentes tipos de inflorescencias por ejemplo, cimosas es propio de las secc. Odoratae, Nipenses, Calophyllae y Lindenianae con la peculiaridad que las tres primeras tienden a ser cimosocorimbosas a veces algo capituliformes en Odoratae y Nipenses; pero pauci- o multiflora en la primera y paucifloras (1-3) en la segunda. Calophyllae a veces tiende a ser paniculada o laxamente apanojado-compuestas, las cimas 9-multifloras. Las cimas simples tri-floras caracterizan a Lindenianae. Rigidae presenta un tipo de inflorescencia no corimbosa, en capítulos bracteados o involucrados, densamente pelosas, sésiles o subsésiles, uni-paucifloras. Racemoso-paniculado es el tipo que caracteriza a la sección Rondeletia, aunque éste patrón varía entre apanojados o laxamente cimosos, cimoso-corimbosos o cimoso-racemosos, usualmente pluriflorоs. El

resto de las secc. presentan inflorescencias en verticilos de 2 e 3 flores, 1-3 flores o flores solitarias, a veces acabezueladas en Pedicellares.

El largo de las inflorescencias es variable y las mismas en general son pelosas variando el indumento de pelositas a tomentosas sobre todo las partes mas jóvenes, éste indumento se va perdiendo con la edad, qunque siempre mantiene un tomento. Podemos señalar diferentes rangos en el largo de los pedúnculos y pedicelos, que permiten caracterizar las diferentes secciones.

Comparativamente resalta que la inflorescencia de Roigella es axilar, pauciflora, cimosa, erecta, con un tomento aplicado; pedúnculos largos y pedicelos muy cortos. Suberanthus tiene inflorescencias terminales, cimosas, en cimas compuestas, tirsoideas, 9-multifloras, glabras, excepto en S. brachycarpus; tienen pedúnculos mayores de un cm y pedicelos mayores de 5 mm.

Desde el punto de vista evolutivo, se aprecia que los tres grupos exhiben al mismo tiempo, caracteres evolucionados o no, en sus taxa.

3.1.6. Flores

La morfología floral es bastante uniforme dentro de Rondeletia. Se distribuyen cerca de la base o del ápice del eje de la inflorescencia, y son flores perfectas, simétricas, infundibuliformes o asalvilladas y varían en tamaño. Roigella tiene flores uniformes en cuanto al tamaño, pero no son perfectas, ni simétricas. Suberanthus, sus flores son pequeñas y coriáceas.

Las flores de Rondeletia presentan brácteas que varían en forma, tamaño y aspecto, algunas son grandes y foliosas como las de Odoratae, Rigidae y Calophyllae y otras son pequeñas y generalmente libres como en el resto de las secciones. Brácteas libres en la parte superior y connadas en la base hasta cerca de la mitad son propias de R. bracteosa Borhidi et Fernández, especie muy característica dentro del género por ésta peculiaridad; involucro de brácteas se presenta en R. rigida Griseb. La mayoría de las brácteas se localizan en número de dos. En todas las secciones se presentan bracteolas. Roigella tiene brácteas grandes, foliosas, en número de dos, igual que sus bracteolas. En Suberanthus las brácteas y bracteolas son pequeñas.

Las piezas florales varían en número de 4-5 (7) meras, en Odoratae el patrón es de 5-7 piezas; 5 en Rigidae, Calophyllae, Rondeletia y Lindenianae; 4 y 5 en Nipenses, Pedicellares y Chamaebuxifoliae; 4 y 6 en Leoninae y 4 en Hypoleucae. En Roigella son 5 y 6 meras y en Suberanthus son 4-meras. Los lóbulos del cáliz son muy variables en forma (triangulares,

triangular-espatulados, deltoideos, oblongo-espatulados, lineales, lanceolados) y tamaño, carácter de gran valor taxonómico en Rondeletia. Se observan libres; en los casos que existe fusión de sus piezas es hacía la base de los sépalos como en algunas especies de Chamaebuxifoliae, Hypoleucae y Nipenses; son persistentes en el fruto, e iguales excepto en algunas especies de la sección Hypoleucae.

Suberanthus y Roigella tienen sépalos libres, persistentes o no en el fruto; en Roigella son iguales, espatulado-triangulares, mayores de 4 mm como en Odoratae, Calophyllae y Lindenianae; en Suberanthus son iguales o desiguales, triangulares a veces espatulado-triangulares a oval-oblongos, son cortos (1-2 mm), tamaño similar a los de Nipenses, y Rondeletia. El resto de las secciones de Rondeletia tienen sépalos entre 2 y 4 mm.

La forma del cáliz en sentido general es globosa para las especies de Rondeletia, con algunas variaciones de un taxon a otro, oblongo-piriforme en Suberanthus y oblongo-obovado con el tubo ensanchado y acostillado arriba, en Roigella. La longitud de la corola se presenta variable en Rondeletia, mayores de 1 cm las tienen generalmente los representantes de las secc. Odoratae, Rigidae, Lindenianae y Leoninae; entre 7 mm y 10 mm es característica en Nipenses y Pedicellares y pequeñas, menores de 6 mm es frecuente en Calophyllae, Rondeletia, Chamaebuxifoliae e Hypoleucae; el indumento también es variable y el tubo de la corola puede ser glabro o pubescente en Pedicellares; antrorso-peloso en Rigidae, Calophyllae, Lindenianae y Leoninae; retrorso-peloso en Odoratae, Nipenses, Rondeletia, Chamaebuxifoliae e Hypoleucae; los lóbulos de la corola también exhiben un tipo pelosidad variable y la forma es generalmente redondeado-obtusa, sin gran significación desde el punto de vista taxonómico. Roigella se caracteriza por tener una corola grande (mayor de un cm), blanca, con pelos aterciopelados retrorso pelosos; lóbulos pelosos en ambas caras, obovado-orbiculares con un desigual, en forma de labelo, carácter derivado, junto a otros primitivos que presenta. Suberanthus spp. tiene corolas pequeñas, hasta 6 mm, coriáceas, carmelitoso-moradas; el tubo glabro excepto en S. brachycarpus que es peloso, lóbulos glabros a glabrescentes y redondeado-orbiculares, Garganta de la corola desnuda en Roigella y Suberanthus; Rondeletia presenta un anillo faucial engrosado.

Rondeletia tiene estambres generalmente en número de 4 ó 5, fijos en la garganta de la corola, ligeramente exertos en algunos casos, insertos en la mayoría, filamentos cortos, estrechos. Estilo delgado, bilobulado, usualmente pubescente, al menos cerca de la base. Roigella tiene 5-6 estambres,

insertos en el tubo de la corola, filamentos breves; estilo bilobulado, hírtulo en la base. En Suberanthus se observan cuatro estambres, insertos sobre la mitad del tubo de la corola, filamentos muy breves, libres. Estilo breve bilobulado, glabro. En los tres casos las anteras se caracterizan por ser oblongo-elípticas, subsésiles y dorsifijas.

3.1.7. Frutos y semillas

Las caracteristicas del fruto y las semillas son muy importantes para la comprensión de Rubiaceae (AIELLO, 1979). Los frutos en los géneros incluídos en Rondeletieae son todos distintos y dentro de los taxa que conforman a Rondeletia s.l. se muestran diferencias tales como:

— Rondeletia s.s. tiene cápsula globosa o subglobosa, cartácea o coriácea, dehiscencia loculicida, con dos celdas bivalvas, glabrescentes (Rigidae, Calophyllae y Pedicellares) a pelosas (el resto de las secciones), de tamaño variable, mayores de 5 mm (Rigidae y Pedicellares), entre 4 y 5 mm (Odoratae, Nipenses, Calophyllae, Lindenianae, Leoninae) y menos de 4 mm (Rondeletia, Chamaebuxifoliae e Hypoleucae).

— Roigella tiene cápsula oblonga a oblongo-piriforme, con dehiscencia septicida, 4 valvas, hirsuta, grandes, mayores de un cm, bilocular.

— Suberanthus con cápsula piriforme, leñosa, coriácea, lepidota, con lenticelas suberosas en la superficie, dehiscencia septicida, 4-valva, grandes, mayores de 7 mm. El tipo de placentación fue otro de los caracteres observados que junto a la forma de la placenta contribuyeron a la separación de los grupos en estudio; se observó que la placenta en Rondeletia s.s. es hemisférica, coriácea, con inserción central en el septo, horizontal o verticalmente sulcada. La placenta de Roigella es coriácea, en forma de escudo, oblonga, con inserción lineal, adherida a todo lo largo del septo. Suberanthus presenta un ovario con placentación basal o sub-basal, la placenta es ascendente, obovada, coriácea u ósea, verticalmente sulcada. En todos los casos los óvulos son numerosos, pero en Roigella están amontonados e imbricados lateralmente, en disposición horizontal; en Suberanthus están verticalmente dispuestos e imbricados, Rondeletia en disposición horizontal.

Rondeletia s.s. tiene semillas diminutas, numerosas, angulosas o fusiformes, a veces apendiculadas o aladas. Roigella las presenta numerosas, pequeñas y son oblongo-lanceoladas bi-apendiculadas o agudas y bi-aladas; las de Suberanthus son discoidales, aladas en toda la periferia, irregularmente laciniadas o fimbriadas, numerosas y diminutas.

3.1.8. Polen

Se examinaron especies que pertenecen a los tres grupos principales que existían dentro de Rondeletia en Cuba y los resultados obtenidos se corresponden con los siguientes tipos:

— Tipo Rondeletia: Granos de polen 3-colporados, prolados, exina diminutamente foveolada.

— Tipo Roigella: Granos 4-5 colporoidados, subesferoidales, exina gruesa, profundamente foveolado-reticuladas.

— Tipo Suberanthus: Granos 3-colporados, reticulo bien desarrollado, con profundas lagunas, grotesco y la superficie rugosa.

Estos patrones se corresponden para Rondeletia con el tipo II citado por AIELLO (1979) y para Suberanthus con el tipo III AIELLO (1979), típico de muchos géneros de la tribu Condamineae, Roigella no se corresponde con ninguno de los patrones citados por esta autora.

En correspondencia con el análisis de los otros caracteres morfológicos estudiados quiero resaltar el valioso aporte de estos resultados que apoyan la diferencia observada entre estos tres grupos.

Además se realizó análisis palinológico a especies de las distintas secciones de Rondeletia que en sentido general mostraron caracteres comunes, como son: granos pequeños, 3-colporados, la ornamentación es finamente foveolada, lo que evidencia que las especies cubanas muestran bastante uniformidad en la morfología del polen. Los resultados se recogen en la tabla 6, junto a otros análisis realizados a géneros aliados y de la tribu Rondeletieae.

3.1.9. Nervadura

El patrón general para la familia Rubiaceae es la presencia de venación pinnada y venas secundarias broquidódromas, tendiendo a formar venas intramarginales, venación terciaria orientada paralelamente a las secundarias (HICKEY, 1975). En las especies examinadas se observó en todos los casos venación camptódroma (broquidódroma), según HICKEY (1973), pero existen diferencias a partir del orden de venación secundario y terciario; mostrando Roigella y Rondeletia venas secundarias y terciarias reticuladas y Suberanthus ramificadas, para los dos órdenes de venación. Los resultados obtenidos se recogen en la tabla 7.

Tabla 6

Tipos de pólenes de algunos taxa

Taxa	NPC (Tipo de apertura)	Diametro (a)	Forma	Ornamentación de la sexina
Acrosynanthus	3-colporado	10x14,5	oblado-esferoidal a prolado esferoidal	finamente foveolado
Acunaeanthus	3-colporado	22x24,5	oblado-esferoidal	granular
Ariadne	3-colporado	10x14,5	sub-oblado a prolado esferoidal	finamente punteado tegilado
Neomazaea	4(5) colporoidado	15x16,5	esferoidal-prolado	reticulada
Rachicallis	4(5) colporado	14-19x13-19	sub-oblado a prolado esferoidal	finamente reticulado
Suberanthus	3-colporado	15-20x18-22	sub-oblado a oblado-esferoidal	reticulos bien desarrollados
Roigella	4(5) colporoidado	18-20x16-19	oblado-esferoidal a sub-prolado	foveolado reticulado
Rondeletia	3-colporado		prolado	diminutamente foveolado
R. chamaebuxifolia	3-colporado	13-15x13-15	sub-oblado a sub-prolado	diminutamente foveolada
R. lindeniana	3-colporado	15-19x15-19	sub-oblado a oblado-esferoidal	finamente foveolado
R. intermixta	3-colporado	13-15x10-13	prolado esferoidal	finamente foveolado
R. leonis	3-colporado	16-19x16-20	oblado-esferoidal a prolado esferoidal	finamente foveolado

3.1.10. Epidermis foliar

El análisis de la misma al microscopio óptico y al microscopio electrónico de barrido para taxa representantes de los tres grupos en estudio, mostraron diferencias significativas en la superficie abaxial y adaxial de las hojas (VALES, MARTÍNEZ, FERNÁNDEZ y DOMINGUEZ, 1989), las que se recopilan en la tabla 8.

Los estomas son en todos los casos del tipo rubiáceo o paracítico; con algunas diferencias en las dimensiones de los mismos (Tabla 8). Los resultados obtenidos para el tipo de estomas eran los esperados (constante) y concuerdan con los que brinda la literatura. Se considera un carácter derivado. También se aprecian variaciones en las características de los tricomas, porque se hallaron pelos simples no septados con el extremo agudo en

Tabla 7

Análisis foliar

Carácter	Rondeletia ssp.	Roigella	Suberanthus
Eje básico de orientación			
1. Curvatura del margen o porte de éste lado de la aserración	cóncavo	cóncavo	cóncavo
2. Organización de la hoja	simple	simple	simple
3. Formas de la hoja			
Lámina completa	simétrica	simétrica	simétrica
Base	simétrica	simétrica	simétrica
Forma	variadas	aovada	oblongo-elip.
Apice	acuminado	redond-obtuso	obtuso a red.
4. Margen	entero	entero	entero
5. Textura	coriácea	coriácea	subcoriácea
6. Peciolo	normal	ausente	normal
7. Venación	pinnada camptódroma (broquidódroma)	pinnada camptódroma (broquidódroma)	pinnada camptódroma (broquidódroma)
	curso de vena primaria recto	curso de vena primaria recto	curso de vena primaria recto
8. Angulo de divergencia (en grados)	agudo moderado (45-55)	agudo estrecho (41-47)	agudo moderado (56-63)
	venas secundarias reticuladas	venas secundarias reticuladas	venas secundarias ramificadas
	venas terciarias reticuladas	venas terciarias reticuladas	venas terciarias ramificadas
9. Areolas	cuadradas formando mallas imperfectas	poligonales formando mallas imperfectas	abiertas formando mallas incompletamente cerradas

S. brachycarpus (grupo tres), el resto de las especies son glabras; pelos simples, incompletamente septados en la especie que representaba al género Rondeletia (grupo uno), y para el grupo dos no obtuvimos resultados, como era de esperarse, porque la especie es glabra (Roigella correifolia). Estos resultados están acorde, en sentido general, con los que brinda la literatura revisada y que caracterizan a Rubiaceae, que tiene pelos de tres tipos principales: septados, uniseriados, compuestos de células separadas; incompletamente septados y septados. La regla general son los pelos rodeados de células epidérmicas dispuestas en forma de roseta en la base del mismo, como en nuestro caso.

Tabla 8

Epidermis foliar

Especies	Superficie adaxial	Superficie abaxial	Pelos	Estomas	
Suberanthus stellatus		MEB Células acompañantes con estrias perpendiculares a las estomáticas M.O. estrias muy finas.		17,1	16,6
Suberanthus yumuriensis	MEB paredes anticlinales hundidas, periclinales granulosas. M.O. paredes anticlinales rectas.	MEB Células acompañantes con estrias perpendiculares a las estomáticas.		7,6	7,5
Suberanthus brachycarpus	MEB paredes anticlinales hundidas y periclinales elevadas. M.O. paredes anticlinales rectas, con engrosamientos.	MEB paredes anticlinales hundidas y periclinales elevadas. M.O. periclinales con estrias.	simples con extremos agudos.	12,3	11,5
Roigella correifolia	MEB paredes anticlinales elevadas, periclinales hundidas. M.O. paredes anticlinales rectas.	M.O. paredes anticlinales rectas, periclinales lisas.		14,6	14,9
Rondeletia odorata	MEB paredes anticlinales elevadas, formando un reticulo; periclinales hundidas. M.O. anticlinales rectas a ligeramente lisas.	MEB paredes anticlinales elevadas y periclinales con ornamentos estriados.	simples simples, septados	9,8 9,8	6, 6,

Los resultados obtenidos son de valor taxonómico porque permitieron corroborar que los nuevos géneros segregados a partir de Rondeletia, poseían caracteres propios desde el punto de vista anatómico que concuerdan con los hallados morfológicamente.

3.2. Integración taxonómica

La visión comparativa expuesta en los epígrafes anteriores constituyeron la base para integrar los resultados desde el punto de vista taxonómico; de tal manera que los caracteres evaluados contribuyeron a separar en grupos el complejo Rondeletia s.l. y a modo de resumen enfatizaré que todos los parámetros concuerdan al diferenciarse en tres patrones marcadamente dife-

rentes que van delimitando a Rondeletia s.s. y conformando los otros dos grupos segregados a partir del criterio amplio con que se concebía el género. Los tres grupos comparten caracteres menos evolucionados y derivados, aunque dada la amplitud del género esto se observa más en Rondeletia; por otra parte Suberanthus exhibe caracteres derivados que en sentido general pudieran considerarse, comparativamente, en mayor número que los que presentan los otros géneros estudiados, como son piezas florales en número de 4, coriáceas, cápsula lenticelada, ápice generalmente redondeado, hojas opuestas y ternadas, lóbulos del cáliz a veces desiguales y cortos, entre otros.

3.2.1. Grupo "correifolia"

Teniendo en cuenta los caracteres analizados se estudiaron las especies del género citadas por ALAIN (1964), para la Flora de Cuba, resultando que a partir de Rondeletia correifolia Griseb. se definieron caracteres diagnósticos que no concuerdan con los señalados para el género objeto de nuestro estudio, tabla 8, lo que posibilitó la descripción de Roigella Borhidi et Fernández, endémico cubano (BORHIDI y FERNÁNDEZ, 1981a). Roigella fue tratado por STANDLEY (1918) como un grupo monotípico denominado "correifoliae" de Rondeletia y ALAIN (1964) siguió el mismo tratamiento. Un reexámen de éste taxon evidenció caracteres anatómicos en el ovario, patrones de placentación y polen diferentes que avalaron la segregación del nuevo género (BORHIDI y FERNÁNDEZ, 1981b), Tabla 9.

URBAN (1898) discutió la ubicación taxonómica de ésta especie y consideró que el taxon debía mantenerse dentro de Rondeletia por tener dehiscencia de la cápsula loculicida. El estudio de cientos de ejemplares de R. correifolia en el herbario y en el campo permitió observar que la dehiscencia de la cápsula de ésta especie es esencialmente septicida, aspecto que enfatiza la separación de éste taxon de Rondeletia.

KELLOG y HOWARD (1987) encontraron el fenómeno del dimorfismo del polen en R. anguilensis descrita por éstos autores; ellos enfatizan que el polen puede ser 3-colpado y 4-colpado en el mismo individuo de una especie dada y señalan que la morfología del polen no puede considerarse como característica fundamental para la separación de géneros afines a Rondeletia, como se tuvo en cuenta en el caso de Roigella y Acunaeanthus. Los resultados obtenidos por KELLOG y HOWARD son interesantes, pero no afectan el valor de los géneros mencionados, porque en ambos la morfología del polen es solo un carácter entre los múltiples analizados y no es el más importante.

Tabla 9

Caracteres diferenciales de los géneros tratados

Roigella	Rondeletia	Suberanthus	Ferdinandusa
5-6 meras	4-5 meras	4-meras	4-meras
Hipantio oblongo-obovado un tubo del cáliz ensanchado y acostillado arriba.	Hipantio globoso	Hipantio oblongo-piriforme con paredes lignificadas.	Hipantio anguloso acostillado, largo
Lóbulos del cáliz largos.	Lóbulos del cáliz variables en forma y tamaño, persistentes.	Lóbulos del cáliz cortos.	Lóbulos del cáliz cortos.
Corola grande, con lóbulos desiguales, uno en forma de labelo, garganta sin engrosar, desnuda.	Corola embudada, tubo glabro por dentro, lobulos imbricados, garganta engrosada, presencia de anillo faucial.	Corola pequeña, coriácea, con garganta estrecha y engrosada, pero con escámulas fauciales por fuera, lamelas lenticeladas.	Corola ligeramente zigomórfica, extendida, garganta sin engrosar.
Estambres 5-6, insertos en el tubo de la corola, filamentos breves.	Estambres 4-5, fijos en la garganta de la corola, ligeramente exertos a veces, filamentos cortos.	Estambres 4, insertos sobre la mitad del tubo de la corola, filamentos muy breves.	Estambres 4, radicados en el medio del tubo de la corola, desiguales, filamentos largos.
Ovario con placentación lineal, longitudinalmente inserta al septo. Placenta oblonga, en forma de escudo.	Ovario con inserción central de la placenta en el septo, horizontal o verticalmente sulcada. Placenta hemisférica, coriácea.	Ovario con placentación basal o sub-basal. Placenta ascendente.	Ovario con inserción de la placenta a todo lo largo de la longitud del septo. Placenta oblongo-lineal.
Cápsula grande, oblonga a oblongo-piriforme, hirsuta, bilocular, dehiscencia septicida.	Cápsula globosa o subglobosa, cartácea o coriácea, con 2 celdas, bivalvas, glabrescentes, dehiscencia loculicida.	Cápsula grande, piriforme, leñosa, coriácea, dehiscencia septicida, 4 valva, lepidota con lenticelas suberosas.	Cápsula oblongo-eliptica u oblongo-lineal, larga acostillada, dehiscencia septicida.
Ovulos numerosos, amontonados, e labricados lateralmente en disposición horizontal.	Ovulos numerosos.	Ovulos numerosos.	Ovulos procos, pedunculados, imbricados.
Semillas comprimidas, apendiculadas a cada lado.	Semillas angulosas, fusiformes a veces apendiculadas o aladas.	Semillas aladas, margen laciniado.	Semillas aladas.
Grano de polen 4-5 colporaidado, profundamente foveolado, sub-esferoidal.	Grano de polen 3- colporado, prolado, exina diminutamente foveoladas.	Grano de polen 3-colporado, con reticulación grotesca, profundas lagunas reticuladas y superficie rugosa.	Grano de polen 3-colporado.
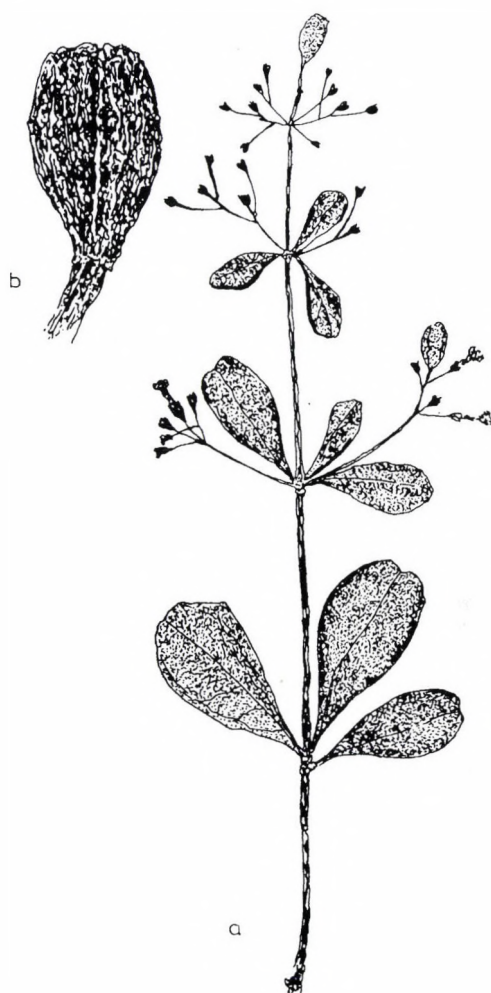
Fig. 3. Suberanthus stellatus (Griseb.) Borhidi et Fernández. a-rama (1x), b-cápsula (7x)

Además, hay que resaltar que en los casos de Roigella y Acunaeanthus no evidenciamos dimorfismo del polen, solo 4 y 5 colpados. Roigella parece se afín a Acunaeanthus (BORHIDI et al., 1980) del Occidente y Centro de Cuba.

3.2.2. Grupo "Rondeletia s.l."

Por otra parte, el estudio de las especies que incluían en su sinonimía los géneros Ferdinandea y Ferdinandusa, brindaron la posibilidad de describir Suberanthus Borhidi et Fernández, de Cuba y la Española (BORHIDI y FERNÁNDEZ, 1981b y 1983), Fig. 3.

Agrupa cinco especies presentes en la flora cubana, muchas de ellas incluídas con anterioridad en otros géneros (ver ALAIN, 1964). URBAN (1898) discute la posición taxonómica de las especies cubanas incluídas en *Ferdinandusa* al plantear que el fruto abre primero loculicidamente. Nosotros observamos cientos de cápsulas abriendo septicidamente desde el primer momento y en ningún caso observamos dehiscencia loculicida. URBAN no estudió la estructura de la placenta.

El género *Ferdinandusa* Pohl. (*Ferdinandea* Pohl.) de la tribu *Cinchoneae*, tiene caracteres buenos que lo diferencian de *Suberanthus*, BORHIDI y FERNÁNDEZ (1981), Tabla 9. BORHIDI (1983) revisa las especies de *Rondeletia* y *Neomazaea* presentes en La Española y comprueba que *R. hincheana* Urb. et Ekm. y *Neomazaea pungens* Urb. pertenecen a *Suberanthus* y describe el subgénero *Moscosoa* Borhidi, endémico de La Española, criterio que comparto. Las investigaciones de epidermis foliar, realizadas por VALES (1983) corroboran que *N. pungens* no pertencene al género donde inicialmente fue ubicada.

Suberanthus por tanto, incluye dos sub-géneros: *Suberanthus* y *Moscosoa*; BORHIDI y FERNÁNDEZ (1983) destacaron los límites del género.

Después de revisar las cinco especies características del subgénero *Suberanthus* y compararlas con otras especies antillanas observamos que presentaban caracteres comunes a las tribus *Cinchoneae* (placenta ascendente, forma y dehiscencia de la cápsula, forma de las semillas, corola coriácea etc.) y *Condamineae* (granos de polen con profundas lagunas y el patrón de la exina) y no a *Rondeletieae*, por eso lo incluímos en la tribu *Cinchoneae* (BORHIDI y FERNÁNDEZ, 1981b).

Rondeletia camagueyensis Standl.; *R. elliptica* Urb. y *R. ternifolia* Urb. (citadas por ALAIN, 1964), descritas a base de material incompleto pertenecen tamibén a *Suberanthus* y pasaron a la sinonimía de *S. brachycarpus*; especie de mayor variabilidad, distribución y vigor aparente, capaz de formar poblaciones hibridógenas (BORHIDI y FERNÁNDEZ, 1983).

Clave para los géneros aliados a *Rondeletia* y los de la tribu *Rondeletieae* en Cuba

1 a Lóbulos de la corola contortos .... ELAEAGIA

  b Lóbulos de la corola imbricados 2

2 a Hojas pequeñas, sentadas, carnosas; cápsula semi-infera .... RACHICALLIS

  b Hojas no carnosas, mayormente más grandes y pecioladas, cápsula infera 3

3 a Cápsula oblonga, alargada o piriforme de dehiscencia septicida 4
b Cápsula globosa o comprimido-elíptica, a veces obovada, mayormente loculicida 7
4 a Inflorescencias terminales en cimas apanojadas, racemiformes, flores rojo-negruzcas, cápsula oblonga a piriforme a menudo lepidota y lenticelada, placenta basal .... SUBERANTHUS
b Inflorescencias axilares o terminales y axilares, flores de otro color, cápsula oblonga o alargada, angulosa o acostillada, placenta central 5
5 a Flores 5-6 meras, corola ligeramente zigomórfica, con un lóbulo labeliforme de margen fimbriado, hojas redondeadas a acorazonadas en la base, semillas apendiculadas o aladas .... ROIGELLA
b Flores 4-meras, actinomorfas, lóbulos de la corola iguales, de margen entero, hojas cuenadas en la base, semillas aladas o apendiculadas .. 6
6 a Flores solitarias, axilares, colgantes, cáliz con tubo alargado, caedizo; corola glabra en la garganta; cápsula linear-alargada, 4-angulosa, semillas pocas, alargadas, apendiculadas .... NEOMAZAEA
b Flores en cimas terminales y axilares, cáliz sin tubo alargado, lóbulos peristentes, corola de tubo largo, pelosa en la garganta; cápsula elíptica, 8-acostillada, semillas numerosas, aladas .... ACUNAEANTHUS
7 a Inflorescencias terminales o terminales y axilares 8
b Inflorescencias axilares 12
8 a Estípulas connadas en un anillo de 4-7 mm de largo, con lóbulos 1-3 cuspidados, cáliz 5-7-mero con lóbulos muy desiguales, corola grande ligeramente zigomorfa, densamente pelosa en la base de los lóbulos, cápsula lateralmente comprimida, sapticida .... JAVORKAEA
b Estípulas libres, lóbulos simples 9
9 a Inflorescencias 1-paucifloras, lóbulos del cáliz carnosos, estambres adnatos cerca de la base del tubo de la corola .... ACROSYNANTHUS
b Inflorescencias multifloras, lóbulos del cáliz no carnosos, estambres adnatos en la garganta de la corola 10
10 a Garganta de la corola hirsuto-pelosa, con pelos amarillos, tubo de la corola peloso por dentro, estípulas grandes................. ROGIERA
b Garganta de la corola lampina estípulas pequeñas 11
11 a Flores 4-meras, tubo de la corola aracnoideo-tomentoso a lampiño por fuera, mayormente peloso por dentro, garganta sin lamelas o anillo faucial, estilo y disco anular glabros, cápsula septicida . ARACHNOTHRYX
b Flores mayormente 5-meras, tubo de la corola estrigiloso-hirsuto por fuera, glabro por dentro, garganta con lamelas o anillo faucial, estilo peloso en la base, disco anular hirsuto, cápsula loculicida . RONDELETIA

12 a Flores 4-meras, tubo del cáliz alargado, lóbulos desiguales por pares, cápsula elíptica, lateralmente comprimida, el exocarpio se abre septicidamente y el meso y endocarpio loculicidamente, semillas una o dos por celdas .... ARIADNE

b Flores 4-5-meras; tubo del cáliz corto o nulo, lóbulos mayormente iguales, cápsula globosa, no comprimida, loculicida, semillas numerosas por celdas .... RONDELETIA

Descripción de Roigella Borhidi et Fernández

Arbusto o pequeños arbolitos, de 2-3 m de altura. Ramas cilíndricas o subangulosas, hírtulas. Estípulas erectas, rígidas, deltoideo-cuspidadas, de 5-7 mm de largo, seríceas. Hojas opuestas, ovales u oblongo-ovales, de hasta 8 cm de largo, la base acorazonada o subacorazonada, subsésiles; ápice redendeado a obtuso, a veces apiculado; margen plano; coriáceas. Inflorescencias axilares, cimosas, paucifloras, brácteas 2, grandes foliosas; pedúnculos largos de hasta 5-8 cm, erectos; flores 5-6-meras, subsésiles; cáliz oblongo-obovado, con el tubo ensanchado y acostillado hacia arriba, lóbulos espatulado-triangulares, mayores de 4 mm; corola blanca, densamente retrorso-hírtula por fuera, mayor de 1 cm, glabra por dentro, lamelas fauciales ausentes, lóbulos de la corola desiguales, uno mayor labeliforme, semiorbiculares a obovado-orbiculares, estambres 5-6, insertos en el tubo de la corola, por debajo de la garganta, filamentos breves; anteras oblongo-elípticas, subsésiles, dorsifijas; estilo bilobulado, hírtulo hacía la base; grano de polen 4-5 colporoidado, globoso, exina gruesa, foveolado reticulado; disco anular densamente velloso; cápsula oblonga a oblongo-piriforme, mayores de 1 cm, hirsuta, bilocular, septicida y 4-valva; placenta coriácea, en forma de escudo, oblonga, con inserción lineal a todo lo largo de la longitud del septo; óvulos numerosos, dispuestos horizontalmente e imbricados; semillas diminutas, oblongo-lanceoladas, biapendiculadas o agudas bialadas, uno de los apéndices o alas muestra una pequeña incisión.

Especie tipo: R. correifolia (Griseb.) Borhidi et Fernández. (Barónimo Rondeletia correifolia Griseb., Cat. Plant. Cub. 1866: 129.) Tipo: WRIGHT 2684, Cuba Occidental.

Descripción de Suberanthus Borhidi et Fernández

Arbusto o arbolitos, de hasta 5(8) m de alto; ramas cilíndricas o angulosas, glabras, raramente seríceas; estípulas triangulares, pequeñas entre

0.5 y 2 mm de largo, coriáceas; hojas opuestas o ternadas, raramente verticiladas, obovadas u oblongas, de hasta 11 cm de largo, coriáceas, generalmente glabras; ápice redondeado a obtuso, a veces agudo, la base cuneada o aguda. Inflorescencias terminales, cimosas, cimas generalmente 9-floras en tirsos multifloros compuestos, pedúnculos mayores de 1 cm y pedicelos mayores de 5 mm; brácteas y bracteolas pequeñas; flores glabras o seríceas, 4-meras; hipantio obovado-piriforme a oblongo-piriforme, pequeño, lóbulos del cáliz iguales o desiguales, triangulares a veces espatulado-triangulares a oval-oblongos, cortos, de 1-2 mm; corola pequeña, de hasta 6 mm, coriácea, carmelitoso-moradas, el tubo glabro por dentro, por fuera glabro o raramente seríceo; lóbulos glabros a glabrescentes, orbicular-obovados, redondeados o truncados, imbricados, anillo faucial estrecho, glabro, lamelas o dentículos fauciales ausentes; estambres 4, insertos sobre la mitad del tubo de la corola, filamentos muy breves, anteras oblongo-elípticas, dorsifíjas, insertas por debajo de la garganta de la corola, estilo breve, bilobulado, glabro, disco anular elevado sobre el ovario, glabro; grano de polen tricolporado, elíptico subesferoidal, exina reticulada, no foveolada, areolas del retículo amplias, rugosas con la superficie rugosa; ovario obovado o piriforme, bilocular, placenta obovada, ascendente, con inserción basal o sub-basal al septo, coriácea u ósea, verticalmente sulcada; óvulos numerosos, dispuestos verticalmente e imbricados; cápsula piriforme, mayores de 7 mm, leñosa, coriácea, lepidota, con lenticelas suberosas en la superficie, septicida, 4 valva; semillas discoidales, aladas en toda su perisferia, irregularmente laciniadas o fimbriadas, numerosas y diminutas.

Especie tipo: Suberanthus neriifolius (A. Rich.) Borhidi et Fernández. (Bason. Exostema neriifolium A. Rich., Sagra Hist. Fis. Pol. Nat. Cuba XI: 7. 1850; sin.: Ferdinandea angustata Wr. in Griseb. Cat. Pl. Cub. 1866: 127.; Rondeletia angustata Wr. in Sauv. Anal. Acad. Habana 6: 122. 1869.; R. calcicola Britt. Bull. Torr. Bot. Cl. 43: 467. 1916.; R. neriifolia Urb. Symb. Ant. 9: 514. 1928. Cuba.)

Relación de especies que pertenecen al género Suberanthus

Subgénero Suberanthus

Suberanthus brachycarpus (Griseb.) Borhidi et Fernández

Toda Cuba; Española: Haití y Santo Domingo

Suberanthus canellifolius (Britt.) Borhidi et Fernández

Cuba. Prov. Holguín. Sierras de Nipe, Cristal y Mícara

Fig. 4. Suberanthus neriifolius (A. Rich.) Borhidi et Fernández.
a — rama (1x), b — flor (10x), c — cáliz (10x), d — boton (10x), e — cápsula (7x)

Suberanthus neriifolius (A. Rich.) Borhidi et Fernández

Cuba. Provs. P. del Rio, Habana, Matanzas, Villa Clara, Sancti-Spíritus e Isla de la Juventud

Suberanthus stellatus (Griseb.) Borhidi et Fernández

Cuba. Provs. Holguín y Guantánamo. (S. de Moa, Cuchillas de Toa y Baracoa, Monte Líbano, S. de Guaso)

Suberanthus yumuriensis (Britt.) Borhidi et Fernández

Cuba. Prov. Guantánamo. Valle del Río Yumuri, al Este de Baracoa

Subgénero Moscosoa

S. hincheanus (Urb. et Ekm.) Borhidi

La Española: Haití, Santo Domingo

S. pungens (Urb.) Borhidi

La Española: Haití

Descripción de Rondeletia L. s.s., emend. Borhidi et Fernández

Arbustos o pequeños arbolitos, ramàs cilíndricas o cuadrangulares, glabras, glabrescentes a pelosas, el tomento a veces ferrugíneo o albo, el que se pierde con la edad; estípulas de tamaño variable (2-10 mm), seríceas o no, triangulares, anchamente triangulares, cuspidadas o subuladas, deltoideas, erectas o no, a veces caedizas; pecíolos de hasta 10 mm, a veces subnulos; hojas aovadas a ovales, oval-oblongas, obovado-oblongas, oblongo-lanceoladas, obovado-elípticas, elípticas, elíptico-lanceoladas, orbiculares a suborbiculares, obovado-orbicular, de 0.5-12 cm por 0.5-10 cm, atenuadas, agudas, obtusas o acorazonadas en la base; acuminadas, agudas, cuspidadas, apiculadas o mucronadas, obtusas o redondeadas en el ápice; coriáceas a membranáceas; glabras, glabrescentes, pelosas o escabrosas en el haz; el envés muy peloso, pubescente o glabro y estrigoso o glabrescente en los nervios, a veces con barbas visibles en las axilas de los nervios primarios, nervios laterales de 2-7 pares, a veces muy prominentes en el envés, regularmente inconspicuos en el haz; margen plano a muy revoluto. Inflorescencias pauci-multifloras, axilares y/o terminales, cimosas, cimoso-corimbosas, a veces capituliformes, otras apanojadas a laxamente cimosas o cimoso-racemosas, también en cimas simples trifloras; brácteas pequeñas o grandes foliosas, libres o connadas en la base, pedúnculos breves o largos, de hasta 0.5-6 cm, pedicelos nulos o hasta 8-10 cm, hipantio globoso, glabro a densamente seríceo, cáliz 4-5-(7) lobulado, lóbulos lineales, lineal-espatulados, oblongo-espatulados, triangulares, triangular-lanceolados, oblanceolados, de 0.5-1 cm de largo, redondeados o agudos, glabros o pelosos; corola generalmente embudada o asalvillada, 4-5-(6-7) lobulada, usualmente blancas o rosadas a veces roja-naranja-salmón; tubo de la corola generalmente glabro por dentro, hirsutico a antrorso o retrorso peloso por fuera, de hasta 3-(5) cm, la garganta desnuda cubierta por lamelas anulares, escamas o dentículos; lóbulos imbricados, glabros o pelositos, redondeados, de hasta 5-(7) mm de

largo; estambres 4 o 5, insertos en la garganta o en el tubo de la corola, filamentos cortos; anteras subsésiles, dorsifijas, oblongo-elípticas. Disco anular hirsuto o velloso. Estilo bilobulado, usualmente pubescentes, al menos cerca de la base. Granos de polen tricolporados, prolados, exina diminutamente foveolada. Placenta hemisférica, coriacea, con inserción central al septo, horizontal o verticalmente sulcada, óvulos numerosos. Cápsula globosa o subglobosa, de hasta 1 cm, glabrescente o pelosa, cartáceas o coriáceas, con dos valvas, loculicidamente dehiscente. Semillas angulosas o fusiformes, a veces apendiculadas o aladas, diminutas.

3.2.3. Grupo "Rondeletia" s.s.

La revisión de todas las especies citadas por ALAIN (1964), auxiliada por las diferentes técnicas y métodos enunciados y discutidos en epígrafes precedentes, confirmaron para Cuba un elevado número de especies que pertenecen a Rondeletia. Las mismas se agruparon para su estudio en 10 grupos las que se citan en el epígrafe 3.1. y se describieron según los resultados obtenidos y expuestos en 3.1.1. a 3.1.10; como se destacó GRISEBACH (1864) y STANDLEY (1918) subdividieron el género en secciones pero las mismas resultaron complejas y heterogéneas e incluían errores en la delimitación y comprensión de los taxa, de ahí que en el avance de ésta investigación se decidió proponer las nuevas secciones, bajo un criterio taxonómico mas práctico y lógico, que incluyeran tanto a representantes cubanos como a sus afines no representados en la Flora de Cuba; tres de ellas mantienen el nombre propuesto por STANDLEY (1918), pero en todos los casos se reestructuraron y definieron sus caracteres de diagnosis por agrupar especies con caracteres no homogéneos, por no llevar el nombre de la especie que la tipifica en algunos casos y por no estar representadas en ellas todas las especies cubanas; ocho de las secciones presentadas por él actualmente la conforman especies que pertenencen a géneros revalidados o nuevos, como son:

Grupo	Género	Grupo	Género
Amoenae	Rogiera	Stellatae	Suberanthus
Leucophyllae	Arachnothryx	Hondurenses	Javorkaea
Laniflorae	Arachnothryx	Tinifoliae	Acunaeanthus
Calycosae	Arachnothryx	Correifoliae	Roigella

Lo que confirma el concepto amplio al concebir el género, que lo siguieron distintos botánicos al estudiar las floras de diferentes paises y Cuba no fue una excepción en éste aspecto; ejemplo lo constituyen Roigella y Suberanthus descritos a partir de taxa incluídos en Rondeletia.

Aqui se presenta la descripción de Rondeletia donde se compilan los caracteres de cada una de las secciones así como las especies cubanas o no que la conforman y las provincias donde se han localizado. La tabla 10 resume los caracteres diagnósticos.

De forma general quiero exponer que las especies que pertenecen a Rondeletia s.s. forman un gran complejo dada la variabilidad morfológica que presentan, incluyendo especies con:

a — hojas grandes, glabras o no, brillantes o no; inflorescencias terminales o axilares excendiendo o no la longitud de las hojas, pauci o multifloras,

b — hojas pequeñas, glabrescentes a pelosas, brillantes o mates, con inflorescencias paucifloras (1-3) o flores solitarias terminales.

Algunos ejemplos ilustrarán lo expuesto; el complejo alaternoides (actualmente definido en la sección IV) pertenecía a un grupo que incluía, entre otras a R. alaternoides A. Rich., R. myrtacea Standl. ex Britt., R. calophylla Standl. ex Britt., R. subglabra Krug et Urb. y R. ekmanii Standl. ex Britt., especies de distribución restringida a las provincias orientales, donde se destaca R. calophylla como una buena especie con caracteres bien definidos, las restantes han sido confundidas con frecuencia; R. alaternoides a pesar de presentar caracteres bien marcados STANDLEY (1918) y ALAIN (1964) no la describieron con exactitud e introdujeron errores en su concepción al citarla como 1-3-flora, siendo multiflora (URBAN, 1923) y la condujeron por ese señalamiento a la afinidad con R. pachyphylla Krug et Urb. (secc. V), sin presentar caracteres afines; en realidad muy cercana a ella es R. myrtacea, que presenta variaciones en la morfología de los lóbulos del cáliz. También forman parte de la secc. IV las nuevas especies descritas por FERNÁNDEZ y BORHIDI (1985), como R. galanensis, fig. 9 y R. lucida, afines entre sí. Por otra parte R. pachyphylla es afín a R. peduncularis A. Rich. (secc. V), fig. 6; de la que difiere fundamentalmente por sus estípulas connadas en la base, hojas pecioladas, ovales a oval-elípticas u oblongas, ápice redondeado a obtuso, margen engrosado y revoluto, lóbulos del cáliz oblongo-espatulados, redondeados a obtusos; lóbulos de la corola oblongo-redondeados y cápsulas mayores, además por que prefiere lugares húmedos. Son vicariantes, se sustituyen una a la otra geográfica- y cenológicamente en el Oriente y Occidente de Cuba, respectivamente.

R. peduncularis crece en matorral xeromorfo espinoso sobre serpentina, de Pinar del Río. R. pachyphylla s.s. (non Alain) es una especie hidrofita,

Tabla 10a

Caracteres diferenciales de las secciones de Rondeletia L.

Secciones	Hábito arbustivo o pequeños arbolitos	Estípulas	Textura	Margen	Pecíolos	Forma	Tamaño
1 Odoratae	·	Triangulares a anchamente triangulares, cuspidadas, subuladas o acuminadas	coriáceas a cartáceas	mayormente recurvo	sésiles a pecioladas (0-7 mm)	aovadas a ovales oval-oblongas, obovado-oblongas, obovado-elípticas	Variable
2 Rigidae	·	aovado-lanceoladas	coriáceas	recurvo	largos (4-7 mm)	aovadas a oblongo-elípticas	grandes mayores de 4,5 cm
3 Nipenses	·	deltoideas	coriáceas a subcoriáceas	generalmente recurvo	largos (4-7 mm)	ovales, oval-oblongos, aovado-ovales	medianas 2-4 cm
4 Calophyllae	·	Triangulares a anchamente triangulares, cuspidadas, subuladas o acuminadas	subcoriáceas	plano a subrevoluto	largos hasta 8 mm	elípticas a oblongo-elípticas, aovadas, ovales a obovadas	grandes mayores de 4,5 cm
5 Pedicellares	·	Triangulares a anchamente triangulares, cuspidadas, subuladas o acuminadas	coriáceas a cartáceas	plano a subrevoluto	cortos 0,5-3 mm	ovales, oval-oblongas, oblongo-elípticas	medianas 2-4 cm
6 Rondeletia	·	triangulares	coriáceas a membranáceas	plano a subrevoluto	largos hasta 8 mm	elípticas, elíptico-oblongas a elíptico-lanceoladas	grandes mayores de 4,5 cm

7 Lindenianae	·	largo-cuspidadas	subcoriáceas	plano a subrevoluto	cortos 0,5-3 mm	mayormente obovadas	medianas 2-4 cm
8 Leoninae	·	Triangulares a anchamente triangulares, cuspidadas, subuladas o acuminadas	coriáceas a subcoriáceas	generalmente recurvo	largos hasta 8 mm	aovadas a elípticas, ovales u obovadas	grandes mayores de 4,5 cm
9 Chamaebuxifolia	·	Triangulares a anchamente triangulares, cuspidadas, subuladas o acuminadas	coriáceas a subcoriáceas	generalmente recurvo	largos 4-7 mm	obovadas, elípticas a elíptico-oblongas, oblanceolado-oblongas	medianas 2-4 cm
10 Hypoleucae	·	variables (todos los tipos espuestos)	coriáceas a subcoriáceas	recurvo	cortos 0,5-3 mm	ovales a suborbiculares, oval-oblongas, oblongas a obovado-oblongas, obovado-elípticas y elíptico-oblongas	pequenas menores de 2 cm

Tabla 10b

Caracteres diferenciales de las secciones de Rondeletia L. (continuación)

Secciones	Apice	Base	Indumento	Posición	Número de flores	Tipo de inflorescencia	Número de piezas florales
1 Odoratae	cuspidadas a cortamente acuminadas o redondeadas	obtusa a acorazonadas o subacorazonadas	glabras o escabrosas haz	axilar a veces terminales	variables	cimoso-corimbosas algo capituliformas	5-7
2 Rigidae	agusos a obtusos	estrechas a obtuso-redondeadas	glabras a con pelos antrorsos	axilares	uni o pauci-cifloras	capitulos bracteados	5
3 Nipenses	obtuso-agudos a redondeados	·	glabras o glabrescentes haz	terminales	1-3 flores	cimoso-corimbosas algo capituliformes	4-5
4 Calophyllae	·	obtusa a subacorazonadas	glabras	·	multifloras	paniculadas o laxamente apanojado-compuestas	5
5 Pedicellares	·	estrechas a obtuso-redondeadas	glabras o glabrescentes haz	·	uni paucifloras	a veces acabezueladas	4-5
6 Rondeletia	agudos a obtusos	cuenadas o estrechas	·	axilares	usualmente multifloras	generalmente racemoso-paniculadas	5
7 Lindenianae	·	·	glabras o con pelos antrorsos	·	1-3 flores	cimas simples	5
8 Leoninae	acuminados a agudos	·	variable	·	uni o paucifloras	verticilos de 2-3 flores	4-6
9 Chamaebuxifolia	agudos a obtusos	estrechas a obtuso-redondeadas	glabras o glabrescentes haz	·	1-3 flores	verticilos de 2-3 flores	4-5
10 Hypoleucae	obtuso-agudos a redondeados	·	generalmente tomentosas en ambas caras	terminales y axilares	1-3 flores		4

Tabla 10c

Caracteres diferenciales de las secciones de Rondeletia L. (continuación)

Secciones	Flores Indumento	Flores Tamaño	Flores Brácteas	Lóbulos del cáliz Forma	Lóbulos del cáliz Tamaño	Libres	Cápsula Forma	Cápsula Indus.	Cápsula Tamaño
1 Odoratae	retrorso pubescente por fuera	grandes o pequeñas mayores de 1 cm	grandes y foliosas	espatuado-triangulares	grandes mayores de 4 mm	x	globosa	pelosas	4-5 mm
2 Rigidae	antrorso pelosas	grandes mayores de 1 cm	grandes y foliosas en involucros	espatulado-triangulares u oblongo-espatulados	medianas 2-4 mm	x	deprimido globosa a subglobosa	glabrescente	> 5 mm
3 Nipenses	retrorso pelosas por fuera	medianas 7-10 mm	pequeñas	aovado-deltoideos o triangular-espatulados	pequeños 1-2 mm	libres o no	globosa	x	4-5 mm
4 Claophyllae	antrorso pelosas a pelositas por fuera	medianas hasta 6 mm	grandes y foliosas	oblongo-espatulados	grandes > 4 mm	libres	globosa a subglobosa	x	4-5 mm
5 Pedicellares	glabra o pubérula por fuera	medianas a pequeñas 7-10 mm	pequeñas	estrechos oblongos u oblongo-elípticos, lanceo-lineales	medianos o pequeños 2-4 mm	x	globosa	x	>5 mm
6 Rondeletia	glabrescentes o retrorso pelosas por fuera	usualmente pequeñas hasta 6 mm	x	deltóideos-triangulares	pequeños 1-2 mm	x	x	pelosa	<4 mm
7 Lindenianae	antrorso pubescente por fuera	grandes > 1 cm	x	oblongo-espatulados o lineal espatulados	grandes > 4 mm	x	x	x	4-5 mm

Tabla 10c (continuación)

Secciones	Flores Indumento	Flores Tamaño	Flores Brácteas	Lóbulos del cáliz Forma	Lóbulos del cáliz Tamaño	Libres	Cápsula Forma	Cápsula Indus.	Cápsula Tamaño
8 Leoninae	pleositas a antrorso pelosas por fuera	grandes >1 cm	x	alargados, oblongo-espatulados o lineal espatulados	medianos 2-4 mm	x	x	x	4-5 mm
9 Chamebuxi-folia	retrorso pelosas por fuera	pequeñas hasta 6 mm	x	lanceo-lineales	medianos o pequeños 2-4 mm	libres o fusionados	x	x	<4 mm
10 Hypoleucae	retrorso pelosas por fuera	medianas o pequeñas hasta 6 mm	pequeñas libres o no	lineales lineal-espatulados, aovado-orbiculares, oblongos, aovado-ovales a deltoideos y lanceolados	pequeños 1-2 mm	x	x	x	<4 mm
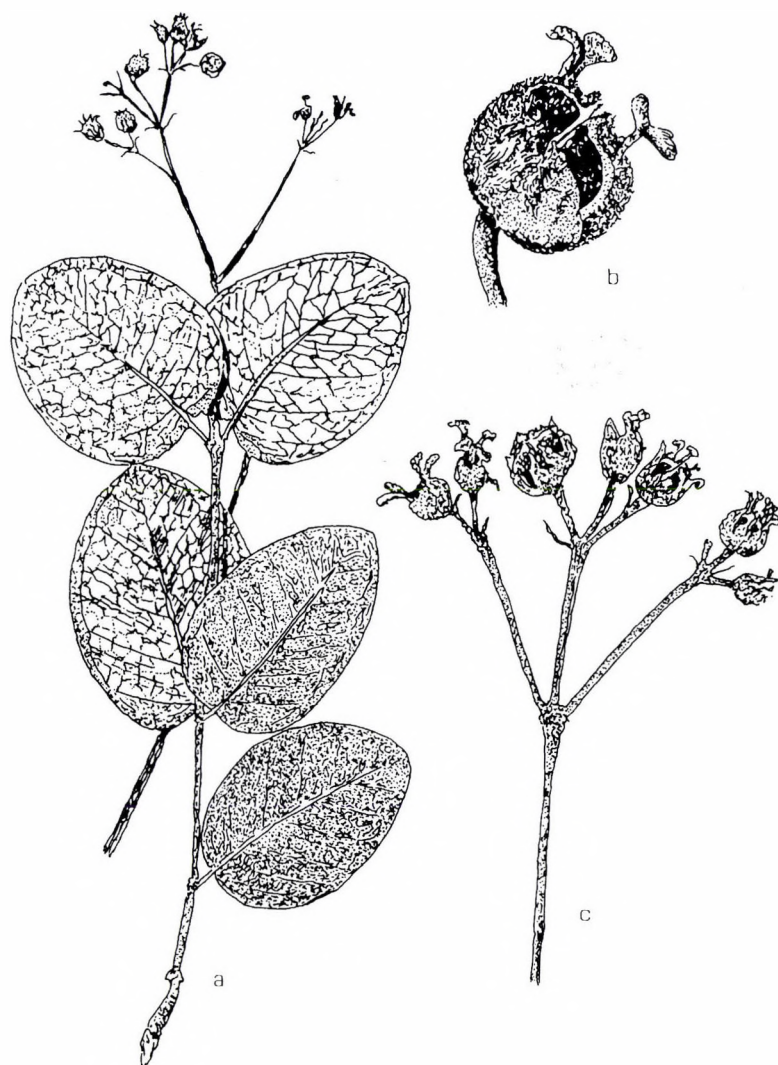
Fig. 5. Rondeletia galanensis Fernandez et Borhidi.
a — rama (1x), b — fruto (3x), c — inflorescencia (3x)

Fig. 6. 1 — Rondeletia peduncularis A.Rich. a — hojas (1x), b — cáliz (10x), c — flor (7x).
2 — Rondeletia pachyphylla Krug et Urb. a — hojas (1x), b — flor (7x)

propia de arroyos en matorral xeromorfo subespinoso sobre serpentina, puede vivir sumergida y presenta dos poblaciones bien definidas en el Norte de Oriente (Sierras de Nipe y Moa). La población típica es la del S de Nipe que es constante en su porte, en el tamaño, forma y textura de sus hojas así como en la nervadura. Estas poblaciones, constituyeron taxa infraespecíficos diferentes que denominamos como: R. pachyphylla Krug et Urb. ssp. pachyphylla y R. pachyphylla Krug et Urb. ssp. myrtilloides Fernández et Borhidi (FERNÁNDEZ y BORHIDI, 1985).

R. odorata Jacq. (secc. I) también tiene caracteres de diagnosis definidos, que la distinguen como genuina integrante de éste género. Se reconoce por sus inflorescencias cimoso-corimbosas, multifloras, mayormente terminales y por sus flores vistosas, naranja-escarlata o rojo-escarlata-salmón; está representada por poblaciones bien definidas que varían según el tipo de suelo y el ecótopo que ocupan, diferenciándose varias subespecies que fueron descritas por FERNÁNDEZ y HERRERA (1983). Es afín a R. naguensis Britt. et Wils. (secc. I), especie que se caracteriza por sus flores en cabezuelas pedunculadas, paucifloras, el indumento albo-estrigoso que presenta en sus ramitas e inflorescencias en contraste con el haz brillante y su cápsula mate subglobosa y pelosa. Es propia de los bosques siempreverde mesófilos de Nagua. Sustituye a R. odorata geográfica y ecológicamente en Cuba Oriental.

R. rigida Griseb., único representante en Cuba, de la secc. II, por ella tipificada, es muy característica por su porte, grandes hojas coriáceas, con domacias y por sus capítulos rodeados por grandes bracteas involucrales, carácter típico de éste grupo que lo define y entre otros lo aparta del resto de las especies y secciones del género.

R. intermixta Britt. (secc. VI), taxon característico, reportado como endémico restringido de La Gran Piedra, colectado por SHAFER (9039) en esta localidad y ampliamente representado en los diferentes herbarios, todos localizados en la zona antes referida; sin embargo al revisar la colección del herbario de Estocolmo (S), existía un ejemplar determinado como R. intermixta, colectado por EKMAN en la Sierra Maestra, Loma Regino, cerca del Turquino, que aunque presenta caracteres propios de ésta especie, no se corresponde con los ejemplares típicos de la especie que aparecen en la localidad clásica, ya que presenta caracteres morfológicos diferenciales en la forma de las hojas, flores y número de nervios laterales, así como en la pelosidad. Por lo expuesto se aprecia que hubo dos poblaciones bien definidas que describimos como: R. intermixta Britt. ssp. intermixta y R. in-

termixta Britt. ssp. *turquinensis* Fernández et Borhidi (FERNÁNDEZ y BORHIDI, 1985). La última población parece haberse extinguido pues no fue posible localizarla nuevamente y sólo contamos con la colección tipo. Presentan vicarianza geográfica, crecen en el mismo tipo de vegetación (bosque nublado), igual tipo de roca (ígneas ácidas) y en altitudes relativamente semejantes, aunque la ssp. *turquinensis* alcanza los 1700 m. s. n. m. Son los únicos representantes de la sección Rondeletia en Cuba, ampliamente representada en las Antillas Menores y Jamaica.

R. lindeniana A. Rich., tipifica la sección (VII) y junto a *R. aurantiaca* Urb. et Ekm., de La Española (únicas representantes de la sección) forman un conjunto caracterizado por sus hojas pequeñas a medianas en verticilos de 4, iguales dos a dos y sus flores largo pedunculadas, con corclas largas.

Todos los miembros de la sección VIII, son cubanos, distribuídos en Cuba Central y Oriental (SAMEK, 1973; BORHIDI, 1986) y *R. leonis* Britt. es la especie tipo, muy afín a *R. monantha* Urb. et Ekm., con la cuál ha sido ampliamente confundida, éste grupo se caracteriza por sus hojas más bien grandes, glabras o glrabrescentes, con inflorescencias axilares pedunculadas, paucifloras. *R. nimanimae* Krug et Urb. las sustituye geográfica y cenológicamente en Cuba Oriental.

Igual que la sección anterior la secc. *Nipenses* (III) es típica cubana, específicamente de Cuba oriental (SAMEK, 1973; BORHIDI, 1985; BORHIDI y MUÑIZ, 1986), sus miembros se localizan en las moñtanas del N de Oriente; *R. subcanescens* Fernández et Borhidi (FERNÁNDEZ y BORHIDI, 1985), anexo 10; es un *taxon* nuevo, afín a *R. lomensis* Urb. de la cuál se diferencia por sus ramitas glabrescentes con la edad, hojas más bién pequeñas, rugosas, alboseríceas y densamente tomentosas por el envés, tubo de la corola de 6-7 mm de largo, pétalos pelosos por dentro.

Por otra parte, el grupo de hojas pequeñas lo conforman las especies incluídas en la sección X *Hypoleucae*, muy característica, compleja, aparentemente similares, disímiles en detalles, que hacen heterogénero éste grupo. Ejemplos: *R. venosa* Wr. in Griseb. a la que se atribuía un tipo de distribución bipolar (Cuba Occidental y Oriental); los estudios esclarecieron que está restringida a P. del Rio (Cajálbana, San Marcos) y que las poblaciones que crecen en la zona Oriental del país algunas corresponden a *R. savannarum* Britt., colectada por SHAFER 1230 en Holguín y que es afín a *R. holguinensis* Urb. colectada por EKMAN 3273 en Cerro del Fraile, Holguín.
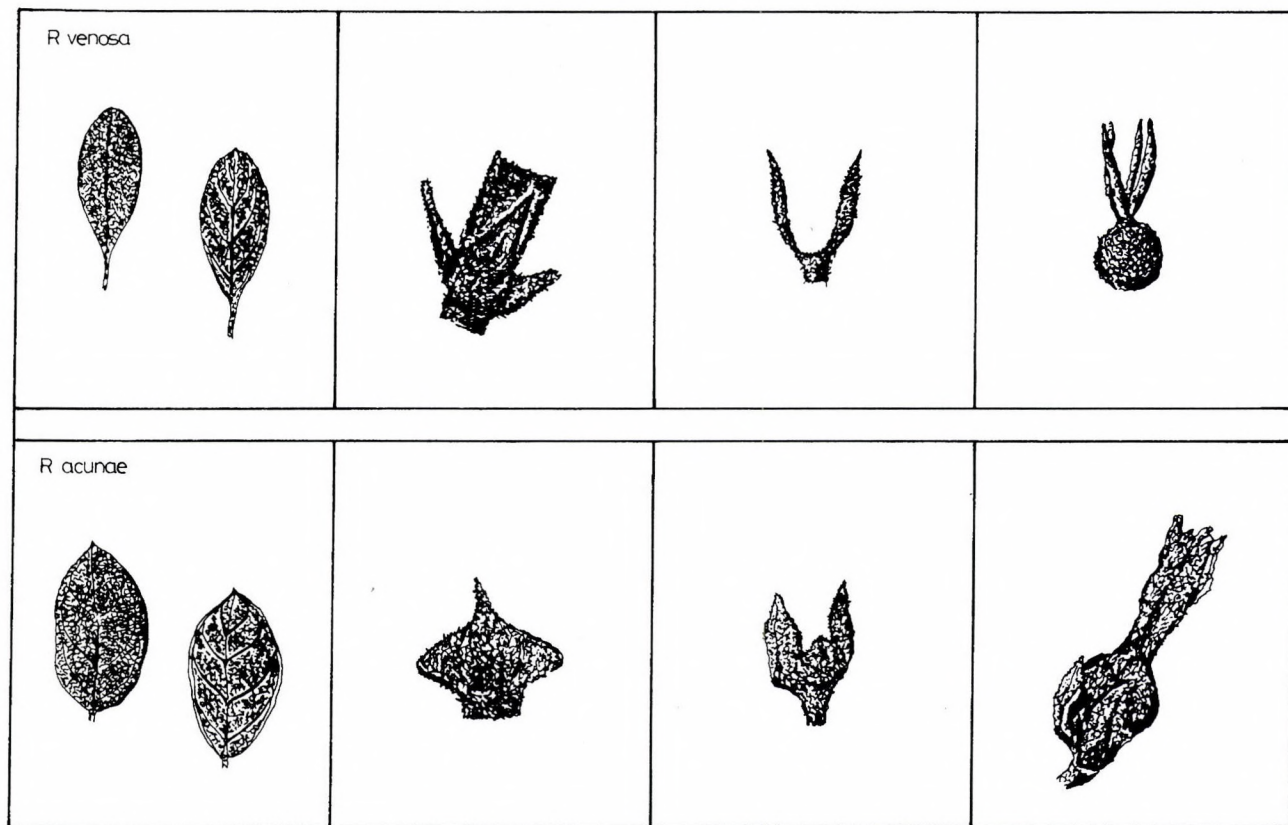
Fig. 7. Caracteres diagnósticos: hojas, estípulas, bracteas, frutos

Las poblaciones con caracteres afines a ésta especie que viven en Moa, correspondieron a un nuevo taxon R. acunae Borhidi et Fernández (FERNÁNDEZ y BORHIDI, 1985), fig. 7, que se distingue de R. venosa por sus estípulas triangulares subuladas, con arista de hasta 2 mm, hojas oblanceoladas, apiculadas en el ápice y mucronadas, algunas algo estrechas hacia la base, otras obtusas; brácteas ninguna o dos, que pueden ser simples o lineales o 3-lobuladas y cáliz deprimido-globoso.

Viven sobre serpentina, R. acunae en matorral xeromorfo sub-espinoso siempreverde sobre serpentinitas; R. venosa en matorral xeromorfo espinoso sobre serpentina. Presentan vicarianza geográfica; están estrechamente relacionadas producto de ésta disyunción geográfica.

R. steirophylla Urb., citada para las montañas del N. de Oriente (ALAIN, 1964) fué colectada por EKMAN (6820) en S. Cristal; bajo éste criterio todas las poblaciones con caracteres similares de ésta zona se determinaron como tal, sin embargo la población que crece en la región de Moa difiere del resto por sus hojas obovadas u orbicular-obovadas o suborbiculares; el haz glabro o muy diminutamente pelosito, brillantes o plegadas; flores sésiles o subsésiles; brácteas dos, triangulares o aovadas, agudas a obtusitas en el ápice, connadas en un involucro tomentoso en la base. Hipantio 4-anguloso; caracteres que la definen como otra especie que describimos y denominamos como R. miraflorensis Fernández et Borhidi (FERNÁNDEZ y BORHIDI, 1985), fig. 8. Crecen en matorral xeromorfo sub-espinoso sobre serpentina. Exhiben vicarianza geográfica.

También afín a R. steirophylla y producto de las nuevas colectas realizadas por el colectivo de trabajo del J.B.N. (HAJB), se describió R. steirophylloides Borhidi et Fernández (BORHIDI y FERNÁNDEZ, 1987), que se reconoce por sus ramitas antrorso-estrigiloso-hirsutas, estípulas cortas, subuladas; hojas pecioladas, glabras y plegadas en el haz, envés con los nervios estrigilosos, antrorso-peloso-tomentosas entre los nervios; brácteas orbiculares u orbicular-espatulados; corola retrorso-estrigilosa por fuera. Se encuentra en Palenque, Guantánamo, en pinares y matorral xeromorfo sub-espinoso sobre serpentina, al SO de Pico Galán, fig. 8. Son vicariantes geográficos.

R. camarioca Wr. ex Sauv. se interpretó bajo un criterio muy amplio determinándose bajo ésta combinación poblaciones que crecen en las antiguas provincias de Oriente, Camagüey, Las Villas, Matanzas y P. del Río (ALAIN, 1964).
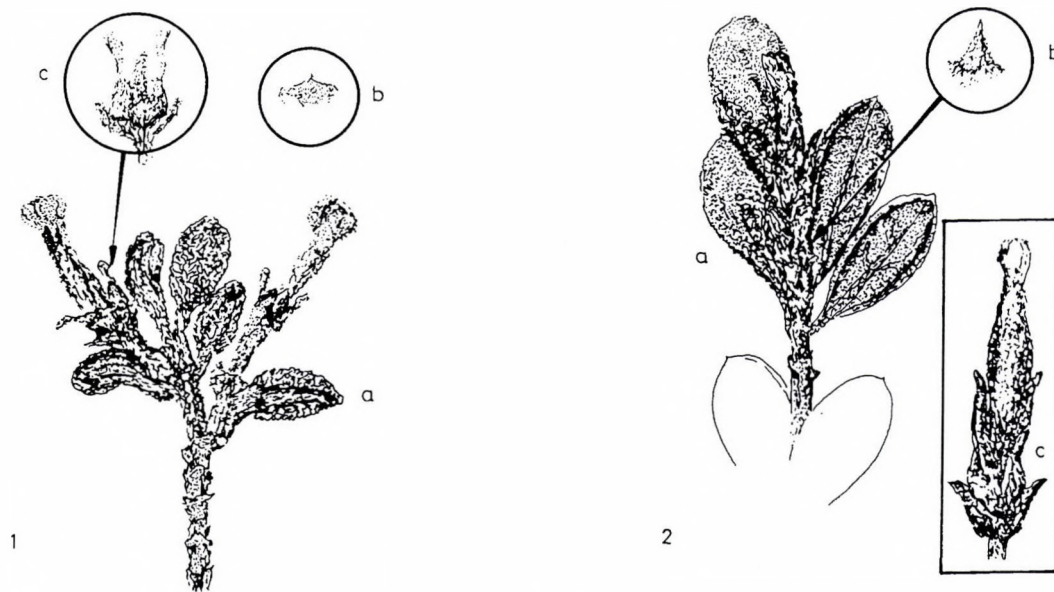
Fig. 8. 1 — Rondeletia steirophylla Urb. a — hojas (1x), b — estípulas (6x), c — caliz (3x).
2 — R. miraflorensis Fernández et Borhidi. a — hojas (2x), b — estípulas (10x), c — cáliz (5x)

Fig. 9. 1 — Rondeletia peninsularis Fernández et Borhidi. a — hojas (2x), b — estípulas (10x), c — capsula (3x), semillas (2000x).
2 — R. camarioca Wr. ex Sauv. a — rama (2x), b — estípulas (10x), c — caliz (5x)

Producto de las investigaciones afirmamos que sólo los taxa que viven en las provincias Habana, Matanzas, Villa Clara, S. Spiritus, Cienfuegos y Camagüey se corresponden con los caracteres de diagnosis dados para éste taxon y con el ejemplar tipo.

No obstante, estas poblaciones muestran variabilidad en cuanto al ancho y largo de las hojas, de las estípulas (Tabla 10) y al indumento ya que las poblaciones de Villa Clara, Cienfuegos y S. Spíritus son muy pelosas en ambas caras de la hoja y en el resto de los órganos vegetativos, sin embargo las de Habana-Matanzas muestran un indumento aplicado que va de glabrescentes a pelosas en el haz, algunas algo brillantes y las de Camagüey son pubescentes, algunas glabrescentes; pudiendo agruparse bajo los mismos caracteres las de Habana-Matanzas-Camagüey (típica) y por otra parte las de Villa Clara-Cienfuegos-S. Spíritus (FERNÁNDEZ y ECHEVARRÍA, 1988), poblaciones que desde el punto de vista taxonómico constituyen variedades.

Algunos ejemplares examinados de Camagüey no presentan diferencias marcadas con R. insularis Britt.; endémica de Cayo Romano. Los especímenes estudiados de P. del Río corresponden a R. venosa.

Las muestras colectadas en las provincias orientales constituyeron un nuevo taxon para la ciencia, que describimos como R. peninsularis Fernández et Borhidi (FERNÁNDEZ y BORHIDI, 1985), fig. 9, que difiere de R. camarioca por tener ramitas amarillo-hirsutas, retrorso pelosas las más jóvenes, hojas agrupadas hacia los extremos de las ramas, elípticas, oblongo-elípticas u oblanceoladas, estrechando hacia la base, pero obtusas, nerviación aparente y hundida en el haz, lóbulos del cáliz 4, triangulares y connados en la base. R. peninsularis se conocía solo de la colección típica (Cabo Cruz, S. de Niquero); al revisar las colecciones del HAJB, lo encontramos representado en el camino del Hondón a la costa, también en prov. Granma. Siempre sobre caliza, en matorral xeromorfo costero y subcostero. Las especies de areal puntiforme, citadas con anterioridad, se localizan en las figs 10 y 11. R. camarioca se localiza en serpentina o en vulcanitas, en formaciones vegetales como matorral xeromorfo espinoso sobre serpentina, presenta un tipo de distribución disyunta, ocupando areales dispersos incluídos en el Sector Cuba-Central (SAMEK, 1973; BORHIDI, 1986), fig. 10.

Ambas especies presentan vicarianza geográfica y ecológica. R. plicatula Urb., especie reportada para el N de Oriente (ALAIN, 1964), después de revisar los ejemplares presentes en los herbarios se definió que los citados para S. del Cristal correspondieron a un nuevo taxon R. bissei Borhidi et
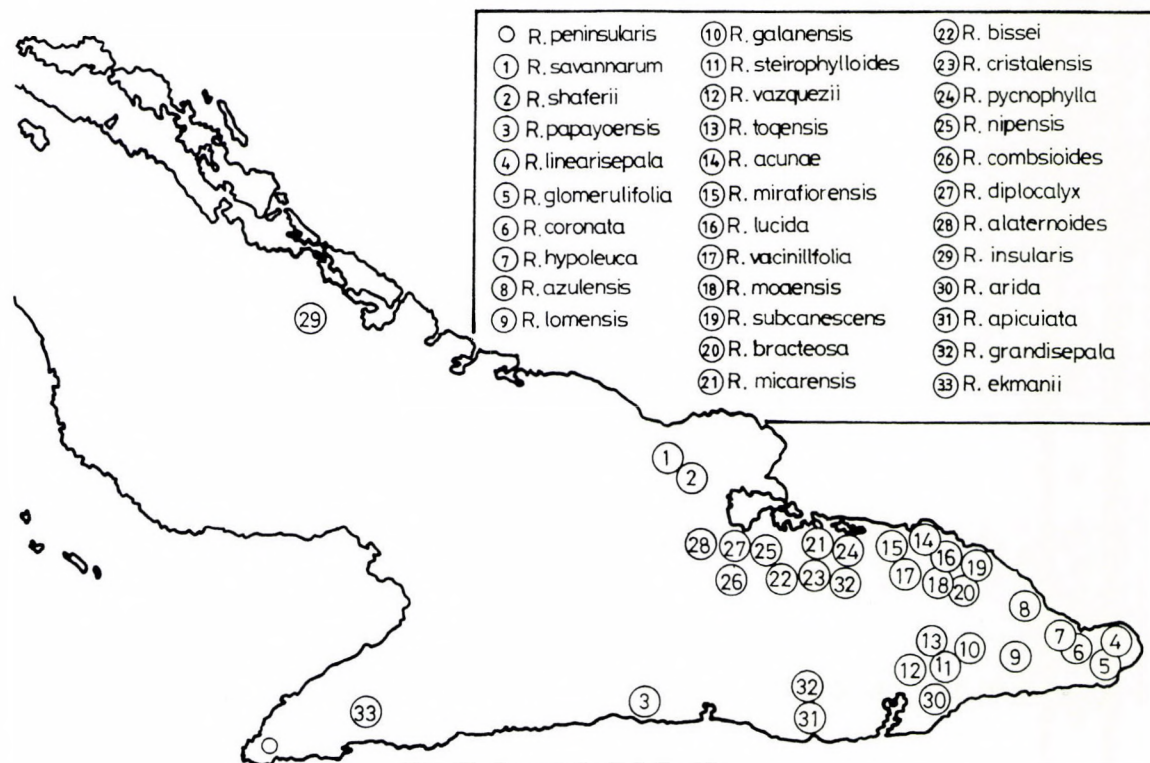
Fig. 10. Esquema de distribución

Fernández (BORHIDI y FERNÁNDEZ, 1987), que difiere de la especie anterior por tener ramitas retrorso pelosas cuando jóvenes, las adultas longitudinalmente estriadas y glabrescentes; hojas anchamente elípticas, densamente albo-tomentosas en el envés, lóbulos del cáliz 4, triangulares, cortos, obtusos o agudos en el ápice, connados en un tubo hasta la mitad. Es propia de matorral xeromorfo sub-espinoso sobre serpentina y su areal es puntiforme. Se diferencia del resto de las especies micrófilas que viven en el n de Oriente, por sus lóbulos del cáliz más cortos. Presenta vicarianza geográfica con relación a R. plicatula que también habita en éste tipo de vegetación.

R. combsii Greenm., con amplia distribución en Cuba Occidental, fue citada por error para las provincias orientales por autores anteriores, ya que éstas poblaciones constituyeron una nueva especie: R. combsioides Fernández et Borhidi (FERNÁNDEZ y BORHIDI, 1985), fig. 10, que difiere de la anterior por sus hojas pecioladas fusiformes, ápice redondeado a obtuso, brevemente apiculado, base largamente estrecha, cuneada, las jóvenes pelosas en ambas caras, las adultas glabras a glabrescentes por el haz, pelositas por el envés, estrigilosas en los nervios; lóbulos del cáliz 4-5, triangulares o deltoideos, muy cortos con el ápice agudo u obtusito, reflejos en la floración. Fué colectada por Ekman, en Holguín (S. de Nipe), sobre caliza. Su distribución es puntiforme. R. combsii vive sobre caliza, en matorral xeromorfo costero y subcostero. Tiene un tipo de distribución disyunta. R. combsii y R. combsioides presentan vicarianza geográfica.

R. papayoensis Fernández et Borhidi (FERNÁNDEZ y BORHIDI, 1985), fig. 10, es otra de las especies descritas producto de ésta investigación, es afín a R. combsioides y muy cercana a R. combsii de las que difiere por tener ramitas retrorso pelosas cuando jóvenes, las adultas escabrositas, hojas algo inequiláteras, estrigiloso-pelosas por el haz, albo tomentosas y estrigilosas en los nervios del envés, lóbulos del cáliz mayormente 4 a veces 5, triangulares o deltoideos, cortos, connados en la base hasta la mitad, obtusos a aguditos en el ápice, reflejos por lo menos en el fruto. Sólo se conoce la colección tipo, depositada en los herbarios de Estocolmo (S) y NYBG (NY), los ejemplares los colecctó Ekman en Papayo (cerca de Sevilla), provincia Stgo. de Cuba. Crece sobre caliza en bosque semideciduo. Tiene un areal puntiforme. Son vicariantes.

R. bicolor Britt. es una especie con caracteres bien definidos, conocida sólo de la colección típica y restringida a los bosques de Ponciano, S. Spíritus, muy cercana a ella en los bosques de Banao se presenta una

población identificada como tal y que responden al criterio, mucho más amplio, concebido para esa especie por ALAIN (1964), el análisis comparativo de las descripciones de Britt. y Alain y el estudio de los ejemplares tipo, evidenciaron que éstos ejemplares presentaban caracteres diferenciales tales como hojas oblanceoladas a oblanceolado-elípticas, glabras, muy rugosas y algo brillantes por el haz, margen muy revoluto, ápice obtuso a veces apiculado, nervios laterales de 4-5 pares; flores 5-meras; lóbulos del cáliz connados en la base hacía un 1/4 de su longitud, lóbulos de la corola albotomentosos; que permitieron describir la especie R. convoluta Fernández et Borhidi (FERNÁNDEZ y BORHIDI, 1985), propia de Lomas de Banao; en realidad es más afín a R. acunae; presentan vicarianza geográfica y cenológica, crecen en bosque pluvial montano.

El grupo Chamaebuxifoliae (Secc. IX) presenta valores intermedios en sus parámetros, pero es más afín a Hypoleucae (Secc. X), entre sus integrantes se destacan R. chamaebuxifolia Griseb. y R. longibracteata Alain, especies muy afines. Han sido ampliamente confundidas por todos los botánicos; ALAIN (1959) describe su especie de un área común a la de R. chamaebuxifolia; ambas conviven en las zonas de Cajálbana, Pan de Guajaibón, Bahía Honda y áreas colindantes, en P. del Río. Sus diferencias son imperceptibles, en cuanto a forma de las hojas, número de piezas florales (que fluctúan entre 4 y 5 en R. chamaebuxifolia), textura de las hojas, margen e inflorescencia, sobre todo las poblaciones de Cajálbana de estas especies. Los caracteres donde se aprecian diferencias marcadas entre ellas son: en la forma de los lóbulos del cáliz y de las brácteas, además el indumento del tubo de la corola; de ahí la dificultad en su diferenciación al colectarlas estériles. De lo antes expuesto y del estudio relaizado por FERNÁNDEZ y ECHEVARRÍA (1987) sobre la variabilidad en estas poblaciones en base a material de herbario, se evidencia la necesidad de ampliar la muestra a analizar y realizar un estudio más profundo en ésta área con la finalidad de definir la variabilidad de la especie R. chamaebuxifolia y la validez de R. longibracteata que se reporta como endémica de Cajálbana, P. del Río y se localiza en matorral xeromorfo espinoso sobre serpentina, mostrando un areal contínuo. R. chamaebuxifolia está representada también en P. del Río, crece en los complejos de mogotes del Pan de Guajaibón (loc. clásica) y en los matorrales xeromorfos espinosos sobre serpentina de Cajálbana y localidades cercanas. Su areal es discontinuo (desde el punto de vista geológico) y prefiere suelos derivados de serpentinitas o de calizas más o menos cársicas. Son vicariantes. Es necesario señalar que ALAIN (1964), plantea que
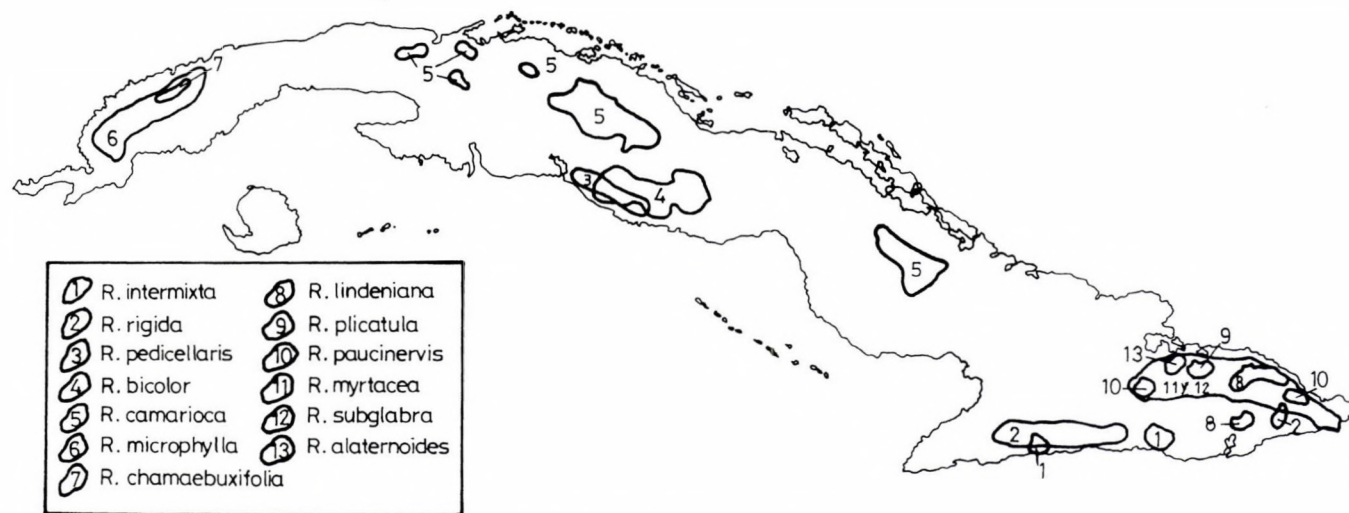
Fig. 11. Esquema de distribución

R. chamaebuxifolia está representada también en Isla de la Juventud (I. P.), pero en nuestras investigaciones no hemos podido observar ningún ejemplar procedente de dicho Municipio Especial que corresponda a la especie antedicha, ahora bién es de suponer que Alain observó la población de Cayo Piedras en sus exploraciones y consideró que ese taxon correspondía a R. chamaebuxifolia; existen en el HAC colectas recientes de esa zona (MONCADA, 1974 y GARCÍA, 1988) que a simple vista pueden ser confundidas con el taxon en cuestión, al estudiarlas detenidamente correspondieron a R. combsii. Colectas anteriores procedentes de Isla de La Juventud de éste género, no se han localizado en herbarios cubanos ni extranjeros. Además, por la diversidad de muestras presentes en las láminas de la colección tipo de la especie discutida, que exhibe en los diferentes herbarios números distintos y material mezclado con otras especies, se hace necesario la lectotipificación de la misma.

Tres de las especies descritas por BORHIDI y FERNÁNDEZ (1987), pertenecen a ésta sección: R. arida, R. bracteosa y R. tubulosa; otra fue descrita por FERNÁNDEZ y BORHIDI (1985), denominándola R. toaensis. El análisis de las descripciones originales, permite afirmar que las realizadas por URBAN (1923--1928) para las especies del género son más completas y acertadas que las de BRITTON (1917), STANDLEY (1918) y ALAIN (1964). Se hicieron las correcciones pertinentes, se complementaron y ampliaron siempre que fue necesario (aspecto que se expondrá en el tratamiento del género para la nueva obra "Flora de la República de Cuba").

El estudio de las claves anteriores, que incluyen especies cubanas, recogidas en las obras de los autores anteriormente citados, evidenció que en las mismas existían imprecisiones en la presentación de algunos caracteres diagnósticos, en el binomio que denomina la especie, excluyen generalmente taxa infraespecíficos, entre otros y se hizo necesario la confección de una nueva clave para las especies presentes en Cuba. También se confeccionaron claves para las secciones y géneros de la tribu Rondeletieae y taxa afines.

ALAIN (1964) presenta 60 especies, posteriormente BORHIDI en BORHIDI y MUÑIZ (1971 y 1975) describe dos más, producto de éste trabajo resultan 65, presentes en la Flora de Cuba; numéricamente la diferencia no es mucha, pero sustancialmente sí, ya que de las 62 anteriormente descritas, sólo se mantienen como válidas 52, porque nueve se excluyeron del género, cinco pasaron a la sinonimía de otras especies; dos de ellas p.p. (por parte); se describieron 21 nuevos taxa, de ellos 15 especies (dos sin publicar) y

cuatro subespecies, que contribuyen al conocimiento de éste complejo grupo; que se encuentra en franca evolución por lo que no se descarta la posibilidad que se describan otros taxa, por ejemplo en Isla de La Juventud, donde existen posibilidades, como lo evidencian las colectas de MONCADA (1974) y GARCÍA (1988) y en el N de Oriente donde Hypoleucae está en pleno desarrollo y adaptación.

A modo de resumen enfatizaré que los caracteres diferenciales, tal como se expuso, hicieron posible segregar Roigella y Suberanthus a partir de Rondeletia s.l.

3.2.4. Análisis complementarios

El Coeficiente de Similitud de Jaccard (Sjs), permitió confirmar que Rondeletia posee una serie de caracteres propios que lo definen como tal y lo apartan del resto de los géneros, aunque los tres presentan cierta afinidad y parecido, destacándose Roigella y Suberanthus por la mayor similitud (0,400); el nivel de similitud de Rondeletia con los otros dos se produce a 0,079. La tabla 3, expone los caracteres utilizados para éste estudio.

Rondeletia comparte caracteres con Roigella en cunato a detalles de las ramas (1), el ápice (7), el estilo (28), inserción de la placenta (32), entre otros, los que fueron expuestos comparativamente en los epígrafes 3.1.1. al 3.1.10, teniendo más afinidad con algunas secciones del grupo de hojas grandes; con Suberanthus sucede otro tanto, presenta caracteres comunes a algunas de las secciones de Rondeletia, como se aprecia en epígrafes anteriores y algunos de los más evolucionados son característicos también de Hypoleucae.

Roigella y Suberanthus presentan caracteres afines (tabla 3) en cuanto a las hojas (5), margen (9), textura (10), ausencia de domacias (11), nervadura (12), tipo de inflorescencia (15), sépalos (23, 24), tamaño de la cápsula (35), etc. Quiero destacar que dado que los caracteres de Rondeletia, son variables (lo que posibilitó su división en secciones), la separación que se observa del mismo con relación a los otros géneros es lógica, ya que son algunos de los caracteres, propios de las diferentes secciones, los que permiten que cada una de ellas independientemente sea más o menos similar a Roigella o a Suberanthus, lo que sugiere un análisis de similitud entre cada una de las secciones con ambos géneros para delimitar cuál de ellas es más afín a uno u otro, aspecto que no era objetivo de ésta investigación para delimitar los caracteres de Rondeletia s.s.

Secc.	10	9	5	3	7	6	8	1	2	4
10		+	+	+	·	-	-	-	-	-
9	0,71		+	+	+	+	·	·	-	-
5	0,67	0,70		+	·	·	+	·	·	·
3	0,67	0,65	0,67		+	·	·	·	·	-
7	0,48	0,70	0,56	0,67		+	+	·	·	·
6	0,42	0,65	0,56	0,56	0,67		+	·	·	·
8	0,40	0,59	0,67	0,48	0,67	0,67		+	-	·
1	0,34	0,48	0,50	0,56	0,56	0,62	0,69		-	·
2	0,30	0,45	0,53	0,47	0,53	0,53	0,44	0,46		·
4	0,22	0,45	0,47	0,33	0,60	0,47	0,59	0,62	0,50	

+ alto
· moderado
- bajo

Fig. 12. Representación del coeficiente de similitud, para las secciones de *Rondeletia*

Producto de las investigaciones, observamos que las secciones que presentan mayor afinidad son *Chamaebuxifoliae* (IX) e *Hypoleucae* (X), que comparten 14 caracteres, le siguen *Odoratae* (I) y *Leoninae* (VIII) con 10, tabla 2; se destaca *Rigidae* (II) por sus caracteres propios, que la definen y apartan del resto de las secciones, aunque no podemos obviar que como todos los *taxa* analizados pertenecen a un mismo género, indudablemente existen caracteres compartidos, entre todas las secciones o entre algunas de ellas, de ahí que ella muestre también caracteres comunes a otras secciones (tabla 2), aunque su mayor afinidad es con el grupo de hojas grandes, sin descartar las posibles relaciones con el grupo de hojas pequeñas. El resto de las secciones tienen distintos niveles de afinidad que pueden apreciarse en la descripción de las mismas (figs 12 y 13).

Un primer análisis de componentes principales (ACP) que incluía todas las variables (27), cuyos tres primeros ejes muestran un 63,1% de la varianza total, permitió definir las variables activas (18), para una segunda valoración. En el mismo se destacan el tamaño de las brácteas, el largo del pecíolo y presencia de indumento en pétalos y frutos como los responsables de la mayor variabilidad entre los *taxa*, caracteres que fueron utilizados entre otros para diferenciar las secciones propuestas y que incluyen todos aquellos que han sido usados tradicionalmente en la delimitación de géneros, secciones y especies, ellos varían significativamente y son característicos para los diferentes grupos combinados con el resto de los caracteres que van
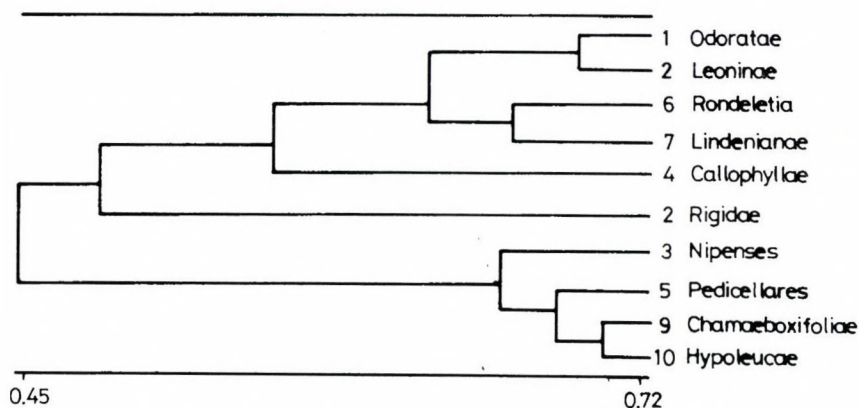
Fig. 13. Dendograma de las secciones de Rondeletia (Sjs)

conformando la individualidad de cada taxon; no obstante puedo señalar, a modo de ejemplo que brácteas grandes, foliosas definen y apartan del resto a Odoratae y Rigidae.

El segundo ACP mostró que los tres primeros ejes (F_1, F_2 y F_3) influyen en la varianza total en un 73,5%, desglosado como sigue, 31,3%, 23,5% y 18,7% respectivamente. Los caracteres que más contribuyeron en la delimitación de los ejes citados son para el primer eje (F_1) forma del ápice, posición y tipo de la inflorescencia e indumento del tubo de la corola, el segundo eje (F_2) quedó definido por el margen de la hoja, número de flores, largo del pedúnculo e indumento de los pétalos; para el tercer eje (F_3), el tamaño de hojas y frutos así como forma e indumento del fruto; éstas definiciones coinciden con el análisis taxonómico realizado, donde éstos caracteres, en sentido general, también contribuyeron de forma relevante a segregar los grupos y corrobora lo antes expuesto, al permitir observar gráficamente (fig. 14) cómo las secciones propuestas se separan unas de otras, lo que avala la selección de los caracteres para definirlas y caracterizarlas como buenos grupos para comprender, delimitar y estudiar Rondeletia. El análisis confirma como Hypoleucae (10), Odoratae (1a, 1b, 1c), Rigidae (2) y Rondeletia (6) son las que se destacan por poseer caracteres menos comunes al resto de las secciones, que aunque son posibles de delimitar taxonómicamente presentan una serie de caracteres que comparten entre ellas, sin excluir que todas entre sí tienen cierto grado de afinidad ya que forman parte de un mismo género.
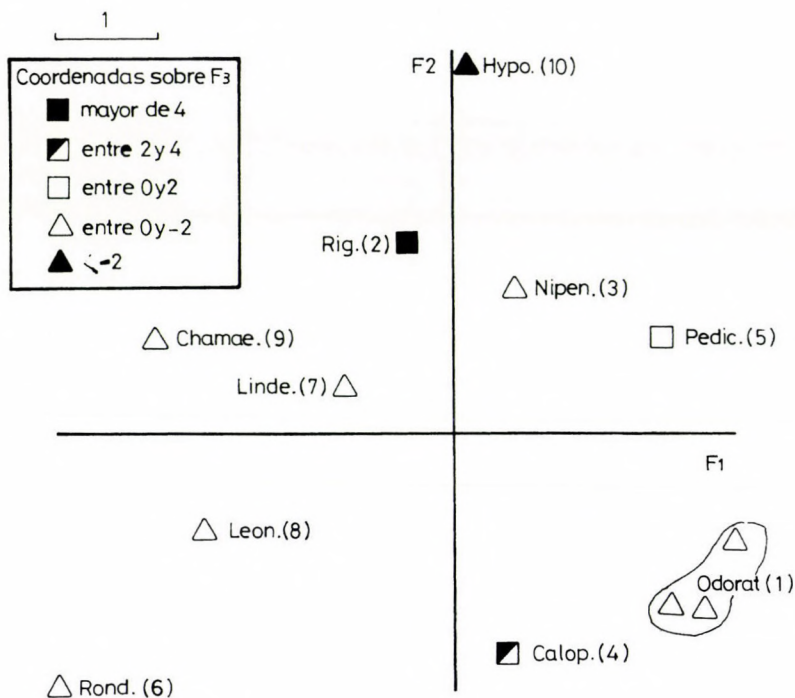
Fig. 14. A.C.P.: Grupos Taxonomicos en Rondeletia

Se confeccionó un dendrograma (fig. 15) en base a las variables activas, el que también manifiesta la separación de las secciones, observándose a Rigidae (2) e Hypoleucae (10) separadas del resto a un nivel de similitud más bajo, seguidas de Rondeletia (6) que está incluída en uno de los dos grupos correlacionados (6, 7, 8, y 9) y (1, 3, 5 y 4) que se destacan también en el dendrograma, manteniéndose la individualidad (por un lado) y la relación (por otro) entre todos los taxa a diferentes niveles de similitud. El coeficiente de similitud de (S_{js}) aplicado a las secciones evidenció resultados que se corresponden los obtenidos bajo el criterio taxonómico clásico, la tabla 2 compila los caracteres utilizados en la aplicación del test; la fig. 12 y el dendrograma (fig. 13) muestran gráficamente el grado de similitud entre los grupos; se definen bien dos grupos, el de hojas grandes (1, 8, 6, 7, 4 y 2) y el de hojas más pequeñas representado por las secciones 10, 9, 5 y 3, quedando la secc. 2 casi separada, delimitando los dos grupos; dentro de los conjuntos definidos se observa que las
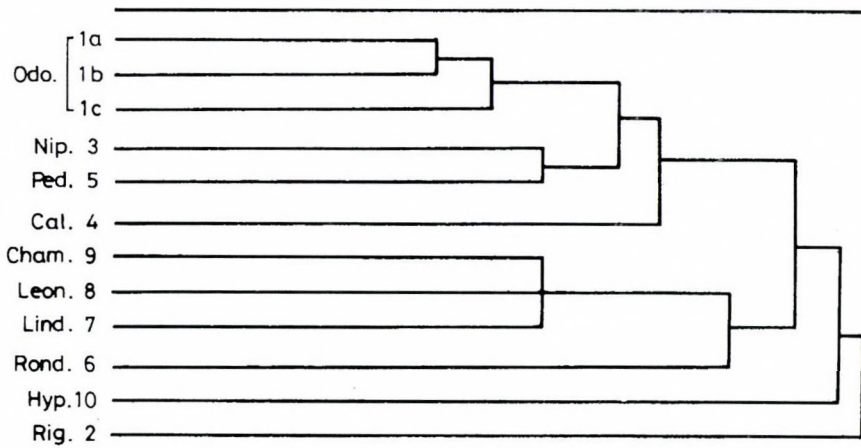
Fig. 15. Dendrograma de las secciones de Rondeletia (A.C.P.)

secciones 9 y 10 tienen el mayor grado de similitud (0,71), siguiendo la 1 y la 8 con 0,69; el resto exhibe coeficientes de similitud menores y diversos entre ellos, el nivel más bajo (0,22) se observa entre las secciones 4 y 10 (fig. 12). En general, las especies de hojas pequeñas presentan entre ellas los mayores coeficientes de similitud; dado por los caracteres que comparten (tabla 2a), forma de la base, domacias ausentes, brácteas pequeñas, indumento similar, forma de la cápsula, etc., aunque es bueno destacar que con el grupo de hojas mayores tienen caracteres semejantes en lo que a textura de las hojas, variabilidad de los lóbulos del cáliz, forma de las estípulas, forma de las ramas (entre otras), se refiere (tabla 10a); el grupo de hojas grandes entre sí muestra relaciones de afinidad variables (figs 13 y 14), y comparten diversos caracteres (tabla 10b).

Por otra parte Hypoleucae posee mayor número de caracteres evolucionados (derivados), como son, el tamaño de sus órganos vegetativos y reproductivos que son pequeños, ramas 4-angulares, pelosas, el indumento se presenta retrorso-peloso en los diferentes órganos, ápice mayormente redondeado, hojas coriáceas, densamente reticuladas, margen recurvo, inflorescencias 1-3 floras, corto pedunculadas, el número de piezas florales reducido a 4, lóbulos del cáliz cortos, espatulado-triangulares y a veces desiguales.

Odoratae, Leoninae y Rondeletia conjugan caracteres menos evolucionados (hojas grandes, piezas florales en número mayor de 4, sépalos iguales, margen plano a veces, inflorescencias multifloras, brácteas grandes, fo-

liosas, membranáceas, etc.) con algunos derivados (inflorescencias pauci- floras en ocasiones, hojas abolladas, brácteas pequeñas, coriáceas, margen recurvo, etc.), aunque en las diez secciones pueden hallarse tanto carac- teres de uno u otro nivel de evolución.

Clave para las secciones del género Rondeletia L. representadas en Cuba

1 a Inflorescencias axilares 6
  b Inflorescencias terminales o terminales y axilares 2
2 a Inflorescencias en cimas corimbosas, multifloras, mayormente terminales, corola pubescente por fuera .... **I. Odoratae**
  b Inflorescencias no en corimbos multifloros 3
3 a Flores en capítulos, ramas e inflorescencias densamente pelosas 4
  b Flores no en capítulos, ramas e inflorescencias glabras o pubescentes. 5
4 a Flores 5-meras, en capítulos bracteados, sésiles, corola antrorso- pelosa .... **II. Rigidae**
  b Flores 4-5-meras, en capítulos o cimas corimbosas paucifloras, bordeados por brácteas pequeñas, mayormente connadas, hojas blanco-tomentosas en el envés, glabrescentes con la edad .... **III. Nipenses**
5 a Flores en cimas 9-multifloras o en panojas laxas a veces compuestas, brácteas inferiores foliáceas, grandes, ramas y hojas mayormente glabras, con nerviación reticulada, muy aparente, el estilo peloso .... **IV. Calophyllae**
  b Inflorescencias 1-3-floras, largamente pedunculadas, nervios laterales poco conspicuos, mayormente reticulados, estilo a veces glabro .... **V. Pedicellares**
6 a Inflorescencias mayormente multifloras, apanojadas o laxamente cimosas o cimoso-corimbosas a cimoso-racemosas .... **VI. Rondeletia**
  b Inflorescentias paucifloras, mayormente pequeñas en cabezuelas sub- corimbosas, densamente cimosas o con flores solitarias 7
7 a Hojas glabras o pelosas en el envés, no tomentosas, nervios laterales poco aparentes, no densamente reticulados 8
  b Hojas densamente grisáceo o blanco-tometosas, nervios laterales sobre- salientes y densamente reticulados en el envés .... **X. Hypoleucae**
8 a Hojas en verticilos de 4, formado por hojas desiguales por pares, pedun- culo largo, lóbulos del cáliz oblongo-espatulados ... **VII. Lindenianae**
  b Hojas no verticilos 9

9 a Inflorescencias con pedunculos más largos de 5 mm, lóbulos del cáliz oblongo-espatulados .... **VIII. Leoninae**

b Inflorescencias con pedunculos cortos, lóbulos del cáliz de otra forma .... **IX. Chamaebuxifoliae**

Descripción de las secciones de Rondeletia y especies que la conforman

I -- Odoratae: Hojas grandes o medianas, cartáceas a coriáceas, pubescentes, algunas domaciadas. Estípulas erectas. Inflorescencia terminal a veces axilares, pauci o plurifloras, usualmente cimoso-corimbosa, las flores 5-(7) partidas; lóbulos del cáliz alargados, a menudo foliáceos, corola larga o pequeña, pubescente por fuera, garganta de la corola glabra. Cápsula globosa, pequeña o mediana. Semillas angulosas o aladas.

Distribución: Cuba y Panamá

Especie tipo: Rondeletia odorata Jacq.

Select. St. Am. Hist. 1763. Vindobonae. Tipo: WRIGHT 2685

Lectotipo: HAC 29780

Localidad: Cuba. Provs.: P. del Río, Habana, C. Habana, Matanzas, Villa Clara, S. Spíritus y Cienfuegos

Especies cubanas en la sección:

Rondeletia naguensis Britt. et Wils.

Bull. Torr. Bot. Cl. 50, 248. 1923. Tipo: LEÓN 10971 NY

Localidad: Provs.: Santiago de Cuba y Granma.

Especies no cubanas que pertenecen a ésta sección:

Rondeletia panamensis DC. Panamá.

II — Rigidae: Hojas grandes, coriáceas, glabras a glabrescentes en el haz, glabras a pelosas en los nervios por el envés; con mechones de pelos en las exilas. Estípulas erectas. Inflorescencias no corimbosas, densamente pelosas, 1-pauci-floras, flores 5-meras, en capítulos bracteados o involucrados, sésiles o subsésiles, lóbulos del cáliz alargados, corola larga, antrorso-pelosa por fuera, glabra por dentro. Cápsula globosa o deprimido-globosa, más pequeña que la corola. Semillas fusiformes.

Distribución: Cuba y Jamaica

Especie tipo: Rondeletia rigida Griseb.

Pl. Wr. in Mem. Am. Acad. n. ser. 8.2.1862. 505. Tipo: 1617 NY

Localidad: Cuba. Prov. Santiago de Cuba.

Especies no cubanas que pertenecen a ésta sección:

R. cincta Griseb. Jamaica
R. glauca Griseb. Jamaica
R. ligulata Urb. Jamaica
R. portlandensis Proctor Jamaica
R. saxicola Britt. Jamaica
R. sylvestris S. Moore. Jamaica

III — Nipenses: Hojas generalmente medianas, coriáces a subcoriáces, blanco-tomentosas en el envés, glabras o glabrescentes en ambas caras, con la edad. Estípulas deltoideas, grandes. Inflorescencias acabezueladas, pedunculadas, bracteosas, axilares o terminales en las ramitas laterales, densamente pelosas, flores en cimas corimbosas, apretadas, paucifloras o en capítulos 1-3 floros, bordeado por brácteas pequeñas mayormente connados en forma de involucro, flores usualmente 4 y 5-meras; lóbulos del cáliz pequeños, corola mediana, retrorso-pelosa por fuera, glabra por dentro. Cápsula globosa, mediana, tomentosa. Semillas aladas.

Distribución: Cuba, endémica
Especie tipo: Rondeletia nipensis Urb.
Symb. Ant. 7.393. 1912. Tipo: SHAFER 3553 NY
Localidad: Cuba. Prov. Holguín. Nipe.
Especies cubanas en la sección:
R. glomeruliflora Alain
Contr. Ocas. 18.1.1960. Tipo: ALAIN 7613 HAC
Localidad: Prov. Guantánamo. Baracoa.
R. lomensis Urb.
Symb. Ant. 7.394. 1912. Tipo: EKMAN 3486 S
Localidad: Prov. Guantánamo. Baracoa.
R. subcanescens Fernández et Borhidi
Act. Bot. Hung. 31 (1--4) p. 168. Tipo: CLEMENTE 6900 HAC
Localidad: Prov. Holguín. Moa.
R. vazquezii Borhidi et Muñiz
Act. Bot. Acad. Sci. Hung. 17:34 1971. Tipo: 27102 HAC
Localidad: Prov. Guntánamo. Cupeyal.

IV — Calophyllae: Hojas grandes, glabras, brillantes y con nerviación reticulada muy aparente, mayormente coriáceas. Estípulas pequeñas. Inflorescencia cimoso-corimbosas a paniculadas, terminales o axilares

excediendo o no la longitud de las hojas, glabras o pubescentes. Brácteas inferiores foliáceas, grandes. Flores 5-meras, en cimas 9-multifloras o panojas laxas a veces compuestas; lóbulos del cáliz pequeños o medianos, corola mediana, apretado-pelosa a pelosita por fuera, glabra por dentro. Cápsula pequeña, globosa a subglobosa, pelosita a glabra. Semillas numerosas, pequeñas, estrechas en ambos extremos.

Distribución: Cuba, endémica

Especie tipo: Rondeletia calophylla Standl. ex Britt.

Bull. Torr. Bot. Cl. 50:48. 1923. Tipo: LEÓN 10741 NY

Localidad: Cuba. Provs.: Santiago de Cuba y Granma.

Especies cubanas en la sección:

R. alaternoides A. Rich.

Sagra 11. 1850, pg. 13. Tipo: LINDEN 2082 P

Localidad: Provs.: Santiago de Cuba y Holguín.

R. ekmanii Britt.

Bull. Torr. Bot. Cl. 51:2. 1924. Tipo: EKMAN 14852 S

Localidad: Prov.: Santiago de Cuba.

R. galanensis Fernández et Borhidi

Acta Bot. Hung. 31 (1--4), pg. 152. 1985. Tipo: ALAIN 3698 HAC

Localidad: Prov.: Guantánamo.

R. grandisepala Alain

Contr. Ocas. 17:1959. 8. Tipo: ALAIN 5698 HAC

Localidad: Prov.: Holguín, Sierra del Cristal.

R. lucida Fernández et Borhidi

Acta Bot. Hung. 31 (1--4), pg. 158. 1985. Tipo: ALAIN 3453 HAC

Localidad: Provs.: Guantánamo y Holguín (Moa).

R. myrtacea Stand. ex Britt.

Bull. Torr. Bot. Cl. 53:464. 1926. Tipo: LEÓN 11966 NY

Localidad: Prov. Guantánamo, Mesa de Prado, Jauco.

R. subglabra Krug. et Urb.

Symb. Ant. 1. 418: 1899. Tipo: LINDEN 2205 P

Localidad: Prov. Santiago de Cuba.

V — Pedicellares: Hojas pequeñas, cartáceas a coriáceas, nervios laterales poco conspicuos, mayormente reticulados; glabras o estrigilosas en los nervios por el envés. Estípulas pequeñas, erectas. Inflorescencias terminales o a veces axilares, mayormente unifloras, raramente 3-floras, flores 4-5-meras, largo pediceladas; lóbulos del cáliz pe-

queños, estrechos; corola pequeña, glabra o pubérula por fuera, glabras en la garganta; estilo a veces glabro. Cápsula pequeña, globosa. Semillas aladas, a menudo apendiculadas en ambos extremos.

Distribución: Cuba y La Española (Haití)

Especie tipo: Rondeletia pedicellaris Wr. ex Sauv.

Anal. Acad. Hab. 6:102:121. 1869. Tipo: Lectotipo Wr. s/n GOET.

Localidad: Cuba. Provs.: Sancti-Spíritus y Villa Clara.

Especies cubanas en la sección:

R. microphylla Griseb.

Cat. Pl. Wer. 127. Tipo: WRIGHT 2691 GOET.

Localidad: Prov. Pinar del Río.

R. minutifolia Urb.

Symb. Ant. 9. 1923. pg. 152. Tipo: EKMAN 10618 S

Localidad: Prov.: P. del Río.

R. pachyphylla Krug. et Urb.

Symb. Ant. 1:419. 1899. Tipo: WRIGHT 2689 GOET.

Localidad: Prov.: Holguín.

R. peduncularis A. Rich.

Sagra 11. 1850. pg. 14. Tipo: SAGRA, s/n P.

Localidad: Prov.: Pinar del Río.

R. pycnophylla Urb.

Symb. Ant. 9. 1923. pg. 151. Tipo: EKMAN 6791 S

Localidad: Prov.: Holguín, Sierra Cristal.

R. shaferi Urb.

Symb. Ant. 7:398. 1912. Tipo: SHAFER 1241 NY

Localidad: Prov.: Holguín .

Especies no cubanas que pertenecen a ésta sección:

R. filisepala Borhidi — Haití

R. mornicola Urb. et Ekman — La Española

R. virgata Sw. — La Española

Dentro de ésta sección se pueden distinguir dos series:

a — Serie Pedicellares: con hojas totalmente glabras, aovadas o acorazonadas, flores 4-meras, lóbulos del cáliz lineales a lanceolados. Incluye R. pedicellaris, R. virgata, R. filisepala y R. mornicola.

b — Serie Pedunculares: con hojas lanceoladas a veces hirsuto-pelosas en los nervios o en el nervio medio; flores 5-meras; lóbulos del cáliz espatulados o espatulado-lanceolados. Incluye las especies cubanas, excepto R. pedicellaris.

VI -- Rondeletia: Hojas grandes, membranáceas o coriáceas, glabras a pubescentes. Estípulas erectas. Inflorescencias axilares, usualmente plurifloras, apanojadas o laxamente cimosas, cimoso-corimbosas o cimoso-racemosas; flores 5-meras; lóbulos del cáliz pequeños, corola usualmente pequeña, infundibuliforme, glabra o pubescente por fuera, la garganta glabra, tubo de la corola alargado, con anillo o dientes fauciales, estilo peloso, estrecho hacia arriba. Cápsula pequeña, globosa. Semillas usualmente aladas o apendiculadas.

Distribución: Cuba, Jamaica, Haití, Puerto Rico y A. Menores.

Especie tipo: Rondeletia americana L.

Sp. Pl. 172. 1753. Tipo: Plum. Pl. Am. pl. 242. f.l., Lam. Tab. Encyc. pl. 162. fl.

Distribución: Jamaica y St. Vicent.

Especies cubanas en la sección:

R. intermixta Britt. ssp. intermixta

Bull. Torr. Bot. Cl. 44:26. 1917. Tipo: SHAFER 9039 NY

Localidad: Prov.: Santiago de Cuba, La Gran Piedra.

R. intermixta Britt. ssp. turquinensis Fernández et Borhidi

Acta Bot. Hung. 31 (1--4), pg. 157. 1985. Tipo: EKMAN 14599 S

Localidad: Prov.: Granma, Sierra Maestra, Loma Regino.

Especies no cunabas que pertenecen a ésta sección:

R. adamsii Proctor	Jamaica
R. arborescens Griseb.	Guadalupe, Martinica, Dominica y St. Vicent
R. brachyphylla Proctor ex Adams.	Jamaica
R. christii Urb.	Haití
R. impressa Krug et Urb.	Jamaica
R. martinicensis Krug et Urb.	Martinica
R. nemoralis Proctor	Jamaica
R. polita Griseb.	Jamaica
R. portoricensis Krug et Urb.	Puerto Rico
R. racemosa Sw.	Jamaica
R. tomentosa Sw.	Jamaica
R. trifolia Jacq.	Jamaica

Quiero destacar que ésta sección con tantos representantes, tiene posibilidades de subdividirse en dos grupos Rondeletia y Racemosae, éste último grupo incluiría las especies R. christii, R. pitreana, R. impressa, R. racemosa, R. brachyanatha y R. intermixta s.l. Este aspecto está en estudio y

por estar incluída sólo una especie cubana en ésta sección se presenta la problemática, pero no profundizo en ello por no ser objetivo fundamental de éste documento.

VII -- Lindenianae: Hojas pequeñas a medianas, en verticilos de 4, formado por hojas desiguales por pares, subcoriáceas, estrigilosas o glabrescentes. Estípulas pequeñas, largo-cuspidadas. Inflorescencias axilares o laterales, en cimas simples 3-floras; flores con pedúnculos largos, 5-meras; lóbulos del cáliz largos, oblongo-espatulados; corola larga, pubescente por fuera, glabra por dentro. Cápsula globosa, mediana y pelosita. Semillas angulosas.

Distribución: Cuba y La Española
Especie tipo: Rondeletia lindeniana A. Rich.
Sagra 11, pg. 13. 1850. Tipo: LINDEN 1834 P
Localidad: Prov. Santiago de Cuba.
Especies no cubanas que pertenecen a ésta sección:
R. aurantiaca Urb. et Ekm. Haití—Española

VIII -- Leoninae: Hojas medianas a grandes, subcoriáceas a coriáceas, glabras o pelositas en los nervios del envés, domaciadas o no. Estípulas pequeñas o medianas. Inflorescencias laterales o axilares con pedúnculos de más de 5 mm, uni-paucifloras, pelosas, flores 4-6-meras, lóbulos del cáliz alargados, oblongo-espatulados; corola larga, pelosita a pelosa por fuera, glabra por dentro. Cápsula globosa. Semillas angulosas.

Distribución: Cuba, endémica
Especie tipo: Rondeletia leonis Britt.
Bull. Torr. Bot. Cl. 44:26. 1917. Tipo: LEÓN 6560 NY
Localidad: Prov. Sancti-Spíritus
Especies cubanas en la sección:
R. nima-nimae Krug et Urb.
Symb. Ant. I: 418, 1899. Tipo: LINDEN 2207 P
Localidad: Provs. Stgo. de Cuba y Granma
R. monantha Urb. et Ekm.
Symb. Ant. 9. 514. 1928. Tipo: EKMAN 16240 S
Localidad: Prov. Sancti-Spíritus

IX -- Chamaebuxifoliae: Hojas medianas, coriáceas a subcoriáceas, glabras o estrigilosas en el envés. Estípulas pequeñas, seríceas. Infloreseen-

cias, axilares, con pedúnculos cortos, 1-3-floras, pelosas; flores 4-5-meras; lóbulos del cáliz cortos; corola pelosa por fuera, glabra por dentro. Cápsula globosa, tomentosa, semillas fusiformes.

Distribución: Cuba, Santo Domingo y Antillas Menores.

Especie tipo: Rondeletia chamaebuxifolia Griseb.

Cat. PL. Cub. 128. 1866. Tipo: WRIGHT 2688 GOET.

Localidad: Prov. Pinar del Río

Especies cubanas en la sección:

R. arida Borhidi et Fernández

Acta Bot. Hung. 33 (1--2), pg. 105. 1987. Tipo: HAJB 20003

Localidad: Prov. Guantánamo. Baitiquirí, camino a Mina del Yeso.

R. bracteosa Borhidi et Fernández

Acta Bot. Hung. 33 (1--2), pg. 109. 1987. Tipo: HAJB 42173

Localidad: Prov. Holguín, Moa

R. cristalensis Urb.

Symb. Ant. 9. 519. 1928. Tipo: EKMAN 15998 S

Localidad: Prov. Holguín (cumbre del Cristal)

R. diplocalyx Urb.

Symb. Ant. 9. 517. 1928. Tipo: EKMAN 15061 S

Localidad: Prov. Holguín (Sierra de Nipe)

R. longibracteata Alain

Contr. Ocas. 17. 9. 1959. Tipo: ALAIN 1367 HAC

Localidad: Prov. Pinar del Río (Cajálbana)

R. micarensis Urb.

Symb. Ant. 9. 519. 1928. Tipo: EKMAN 15932 S

Localidad: Prov. Holguín (Mícara)

R. paucinervis Urb.

Symb. Ant. 9. 518. 1928. Tipo: EKMAN 19151 S

Localidad: Prov. Holguín (Nipe)

R. toaensis Fernández et Borhidi

Acta Bot. Hung. 31 (1--4), pg. 170. 1985. Tipo: ALAIN 3494 HAC

Localidad: Prov. Gunatánamo

R. vaccinifolia Britt.

Bull. Torr. Bot. Cl. 44. 29. 1917. Tipo: SHAFER 4090 NY

Localidad: Prov. Holguín (Moa)

Especies no cubanas que pertenecen a la sección:

R. areolata Urb. Santo Domingo

R. brigandina Urb. et Ekm. Santo Domingo

R. buxifolia Vahl	A. Menores (Monserrat)
R. domatiata Urb.	Sto. Domingo
R. heterochroa Urb.	Sto. Domingo (Barahona)
R. nalgensis Urb. et Ekm.	Sto. Domingo
R. perfae Alain	Sto. Domingo (Rep. Dominic.)

X -- Hypoleucae: Hojas generalmente pequeñas, coriáceas a subcoriáceas, nervios laterales prominentes y densamente reticulado-venosas, blanco-grisáceo tomentosas por el envés, al menos cuando jóvenes. Estípulas generalmente pequeñas, erectas. Inflorescencias terminales o axilares, usualmente 1-3 floras, las flores mayormente 4-meras; lóbulos del cáliz pequeños, brácteas pequeñas; corola mediana, retrorso-pelosa por fuera, la garganta glabra. Cápsula pequeña, globosa. Semillas aladas.

Distribución: Cuba, La Española (República Dominicana)

Especie tipo: Rondeletia hypoleuca Griseb.

Cat. Pl. Cub. 128. 1866. Tipo: WRIGHT 2692 GOET.

Localidad: Prov. Guantánamo (cerca de Baracoa)

Especies cubanas en la sección:

R. acunae Borhidi et Fernández

Acta Bot. Hung. 31 (1--4), pg. 147. 1985. Tipo: B.30259 HAC

Localidad: Prov. Holguín (Moa).

R. apiculata Urb.

Symb. Ant. 9. 554. 1923. Tipo: EKMAN 9229 S

Localidad: Prov. Stgo. de Cuba (Aguadores)

(R. norlindii Urb.)

Symb. Ant. 9. 556. 1923. Tipo: EKMAN 8687 S

Localidad: Prov. Stgo. de Cuba (Aguadores)

R. azulensis Urb.

Symb. Ant. 9. 123. 1923. Tipo: EKMAN 4392 S

Localidad: Prov. Guantánamo (Sierra Azul)

R. baracoensis Britt.

Bull. Torr. Bot. Cl. 44:27. 1917. Tipo: 245 NY

Localidad: Prov. Guantánamo

R. bicolor Britt.

Bull. Torr. Bot. Cl. 44:30. 1917. Tipo: LEÓN 6717 NY

Localidad: Prov. Sancti-Spíritus (Loma de Ponciano).

R. bissei Borhidi et Fernández

Acta Bot. Hung. 33 (1--2), pg. 107. 1987. Tipo: HAJB 30740

Localidad: Prov. Stgo. de Cuba (Mayarí Arriba, Srra. Cristal).

R. camarioca Wr. ex Sauv.
Anal. Acad. Hab. 6: 102. 1869. Tipo: WRIGHT 3579 A
Localidad: Provs. Habana, Matanzas, V. Clara, S.-Spíritus y Camagüey.
(R. gamboana Urb.)
Symb. Ant. 9:520. 1928. Tipo: EKMAN 14989 S
Localidad: Prov. Las Tunas (Gamboa)
R. insularis Britt.
Bull. Torr. Bot. Cl. 44:28. 1917. Tipo: SHAFER 2444 NY
Localidad: Prov. Camagüey (Cayo Romano).
R. combsii Greenm.
Trans. Acad. St. Louis 7:427, pg. 34, 1897. Tipo: Combs. 527 NY
Localidad: Provs. V. Clara, S-Spíritus, Habana, P. del Río
R. combsioides Fernández et Borhidi
Acta Bot. Hung. 31 (1--4), pg. 149. 1985. Tipo: EKMAN 15135 S
Localidad: Prov. Holguín (Nipe).
R. convoluta Fernández et Borhidi
Acta Bot. Hung. 31 (1--4), pg. 151. 1985. Tipo: Luna 865 HAC
Localidad: Prov. Sancti-Spíritus
R. coronata Urb.
Symb. Ant. 9. 153. 1923. Tipo: EKMAN 3561 S
Localidad: Prov. Guantánamo (Baracoa)
R. ingrata Standl. ex Britt.
Bull. Torr. Bot. Cl. 53:465. 1926. Tipo: LEÓN 12415 NY
Localidad: Prov. Guantánamo (Río Jojó, Cajobabo).
R. linearisepala Alain
Contr. Ocas. 17. 2. 1959. Tipo: ALAIN 7679 HAC
Localidad: Prov. Guantánamo (Baracoa, Yumurí).
R. miraflorensis Fernández et Borhidi
Acta Bot. Hung. 31 (1--4), pg. 154. 1985. Tipo: ALAIN 963 HAC
Localidad: Prov. Holguín (Cerro de Miraflores, Moa).
R. papayoensis Fernández et Borhidi
Acta Bot. Hung. 31 (1--4), pg. 165. 1985. Tipo: EKMAN 9306 S
Localidad: Prov. Stgo. de Cuba (Papayo, cerca de Sevilla).
R. peninsularis Fernández et Borhidi
Acta Bot. Hung. 31 (1--4), pg. 166. Tipo: EKMAN 16167 S
Localidad: Prov. Granma (Cabo Cruz, S. de Niquero).
R. plicatula Urb.
Symb. Ant. 9. 154. 1923. Tipo: EKMAN 6024 S
Localidad: Prov. Holguín (Nipe, Río Piloto).

R. potrerillona Urb.
Symb. Ant. 9. 520. 1928. Tipo: EKMAN 18953 S
Localidad: Prov. S.-Spíritus (Pico Potrerillo).
R. rugelii Urb.
Symb. Ant. 7. 397. 1912. Tipo: RUGEL 311 NY
Localidad: Prov. Matanzas
R. savannarum Britt.
Bull. Torr. Bot. Cl. 47:29. 1917. Tipo: SHAFER 1230 NY
Localidad: Prov. Holguín.
(R. holguinensis Urb.)
Symb. Ant. 9:517. 1928. Tipo: EKMAN 3273 S.
Localidad: Prov. Holguín (Cerro del Fraile).
R. steirophylla Urb.
Symb. Ant. 9:155. 1923. Tipo: EKMAN 6820 S
Localidad: Prov. Holguín (Sierra Cristal).
R. steirophylloides Borhidi et Fernández
Acta Bot. Hung. 33 (1--2), pg. 112. 1987. Tipo: HAJB 9495
Localidad: Prov. Guantánamo (Palenque, Pico Galán).
R. susannae Borhidi
Act. Bot. Acad, Sci. Hung. 21 (3--4), pg. 230. 1975. Tipo: 27695 HAC
Localidad: Prov. Pinar del Río (Sierra de la Güira).
R. venosa Wr. ex Griseb.
Cat. Pl. Cub. 128. 1866. Tipo: Wr. 2693 A
Localidad: Prov. P. del Río (San Marcos, Cajálbana).
Especies no cubanas que pertenecen a la sección:

R. berteriana DC.	Santo Domingo
R. brauseana Urb.	Santo Domingo
R. exasperata Borhidi	Rep. Dominicana
R. fuertesii Urb.	Santo Domingo
R. liogieri Borhidi	Rep. Dominicana
R. royenifolia DC.	La Española

Dentro de ésta sección se pueden distinguir siete series denominadas Combsii, Bicolores, Canescentes, Venosae, Hypoleucae, Camariocae e Ingratae, donde se agrupan las especies según sus caracteres afines, para un mejor estudio e interpretación de las mismas.

Tabla 11. Variables utilizadas en el analisis de componentes principales

a) Ramas:
1. Cilíndricas pelosas
2. Cuadrangulares pelosas

b) Dimensiones de las estípulas:
1. 0,5—2,0 mm
2. 2,5—4,5 mm
3. mayor de 5 mm

c) Forma de las estípulas:
1. largo cuspidadas
2. deltoideas
3. triangulares subuladas o acuminadas
4. triangulares
5. aovado — lanceoladas

ch) Largo del peciolo:
1. 0,5—8,0 mm pelosas
2. 0,5—3,0 mm antrorso pelosas
3. 0,5—3,0 mm retrorso pelosas
4. 4,0—7,0 mm pelosas
5. mayor de 8 mm antrorso pelosas

d) Tamaño de las hojas:
1. 1,0—6,0 cm
2. 0,5—1,0 cm
3. 2,0—4,0 cm
4. mayor de 4,5 cm

e) Indumento de las hojas:
1. glabras
2. glabras o con pelos antrorsos en los nervious del envés
3. glabras o glabrescentes en el haz
4. tomentosas

f) Forma del ápice:
1. acuminado o agudo
2. agudo a obtuso
3. obtuso — agudo — redondeado
4. cuspidado a cortamente acuminado o rendondeado

g) Forma de la base:
1. estrechada o cuneada
2. obtusa a acorazonada o subacorazonada
3. estrechada a obtuso-redondeada

h) Margen de la hoja:
1. plano
2. plano o revoluto
3. plano o subrevoluto
4. recurvo

i) Texture de la hoja:
1. cartacea a subcoriacea
2. coriacea
3. membranacea — cartacea —subcoriacea — coriacea

j) Posición de la inflorescencia:
1. terminal
2. terminal y axilar
3. axilar

k) Número de flores:
1. multifloras
2. uni — paucifloras
3. 1-3 floras
4. pauci o multifloras

l) Tipo de inflorescencias:
1. solitaria
2. racemosa — paniculada
3. no corimbosa
4. cimosa
5. acabezuelada

m) Largo del pedúnculo:
1. menor de 1,0 cm
2. 1,0—3,0 cm
3. mayor de 1,0 cm

n) Largo del pedicelo:
1. nulos
2. 1,0—5,0 mm
3. mayor de 5,0 mm

ñ) Tamaño de las brácteas:
1. grandes foliosas
2. pequeñas

o) Número de sépalos y pétalos:
1. 4
2. 4—5
3. 4—6
4. 5
5. 5—7

p) Semajanza entre sépalos:
1. igulaes
2. iguales o desiguales

q) Tamaño de los sépalos:
1. 1,0—2,0 mm
2. 2,0—4,0 mm
3. mayor de 4,0 mm

r) Soldadura de los sépalos:
1. libres
2. libres y connados

s) Forma de los sépalos:
1. lanceo-lineares
2. espatulado-triangulares
3. oblongo-espatulados
4. linear-espatulados

t) Largo de la corola:
1. hasta 0,6 cm
2. 0,7—1,0 cm
3. mayor de 1,0 cm

u) Indumento del tubo de la corola:
1. pubescente
2. antrorso-peloso
3. retrorso-peloso

v) Indumento de los pétalos:
1. glabros o glabrescentes arriba
2. glabros o glabrescentes en ambas caras
3. pelosos en ambas caras

w) Tamaño del fruto:
1. hasta 4,0 mm
2, 2,0—5,0 mm
3. mayor de 5,0 mm

x) Indumento del fruto:
1. glabrescente
2. peloso

y) Forma del fruto:
1. globoso
2. globoso a subgloboso
3. deprimido globoso a subgloboso

3.3. Biogeografía del género Rondeletia

Las evidencias fósiles para la familia Rubiaceae datan del Eoceno Superior (Jan MUELLER, 1981). En América, son pocos los registros fósiles que se atribuyen a la familia, sin embargo, GRAHAM y JARZEN (1969) reportaron que la muestra de polen fósil más antigua corresponde al género Faramea encontrada en sedimentos del Oligoceno de Puerto Rico. Con relación a Rondeletia y taxa afines, hasta el presente, en los estudios paleobotánicos realizados, no se han observado restos del tipo de polen que presentan esos géneros, aunque GRAHAM (1988), señala que BERRY (1918 y 1923b) en estudios de plantas megafósiles reportó restos de Rondeletia del Terciario en general, para Panamá, esto no constituye una evidencia confiable ya que GRAHAM (1988) continúa su análisis resaltando que en la década de 1918—1928 BERRY y HOLLICK estudiaron activamente plantas megafósiles (semillas, frutos, hojas y maderas) del Terciario del Norte de América Latina para reconstruir la historía de la vegetación, pero se ha comprobado que muchas determinaciones genéticas no son confiables y las floras no se han revisado, de ahí que los estudios recientes utilizando microfósiles (polen y esporas), han contribuído a reconstruir la flora del Terciario del N de América Latina con mayor exactitud data la facilidad de conservación de los palinomorfos y en ningún caso han reportado polen del género en estudio (GRAHAM, 1988; GRAHAM y JARZEN, 1969). Es por ello que sin antecendentes es difícil precisar cómo y cuándo Rondeletia llega a Cuba. La familia tiene un origen Gondwanico, RAVEN y AXELROD (1974), GENTRY (1982), éste género aparentemente surge en Las Antillas y está limitado a los Neotrópicos.

KIRKBRIDE (1968) plantea que las dos principales áreas de evolución y distribución del género son México-America Central y Las Antillas y que existe un pequeño centro en el N de Am. del Sur y que panamá corta los complejos México-Am. Central y el Suramericano, teniendo afinidades con ambas.

El concepto amplio concebido para el género por botánicos anteriores contribuía a que estuviera representado en México, Centro América, N de Suramérica, Panamá y Las Antillas, sin embargo los estudios recientes que han reducido la sinonimía del género, revalidando algunos taxa o describiendo otros para la ciencia (como se expone en los epígrafes 1.5 y 3.1. de éste documento), han contribuído a la reducción del área de distribución de Rondeletia y en la actualidad se limita a Las Antillas y Pamamá, no se excluye la posibilidad que producto de la revisión de Rondeletieae en áreas continentales, se corrobore la presencia de Rondeletia en esas tierras. Cuba se destaca por poseer 65 especies, 59% del total todas endémicas, siguiendo en orden decreciente Jamaica (16), La Española (15), St. Vicent y Martinica (2 cada una), Puerto Rico, Dominica, Guadalupe, Monserrat, Anguila y Panamá poseen una cada una, figs 1 y 2. En las Antillas Mayores están representadas 97 especies del total (88%) y las ocho representadas en Las Antillas Menores, constituyen el 7,2%. De acuerdo con esto coincido con parte de la hipótesis de KIRKBRIDE (1968) en cuanto a que Las Antillas es el área de evolución y distribución del género; BORHIDI (1985) considera que Cuba es el centro de evolución secundario para Rondeletia entre otros que destaca, debido a los cambios climáticos y geológicos ocurridos en Las Antillas, sustentado por su hipótesis de que en el Oligoceno Medio y Superior el bloque de La Sierra Maestra y Honduras estuvieron unidos vía El Gran Caimán y el bloque Moa-Baracoa junto a la Española probablemente formaron parte de Honduras--Jamaica--Española y Puerto Rico, vía que fue la ruta principal de inmigración, por tanto los bloques Sierra Maestra y Moa-Baracoa estuvieron unidos al resto de Las Antillas Mayores por un largo periodo de tiempo, en contraposición al resto de los bloques en que estuvo dividida Cuba en éste período y que pertenecen a Cuba Occidental y Central que estuvieron unidos por períodos más cortos a las tierras vecinas.

En la actualidad Cuba se destaca por la concentración de especies que presenta; teniendo en cuenta el principio de "centro de dispersión" enunciado por CAIN (1951) que define que para las especies jóvenes el centro de dispersión es el centro de origen; sugiero la posibilidad que Cuba sea parte del centro primario de evolución, el principal de diversificación y el de dispersión probable de las especies del género, ya que si tenemos en cuenta

los criterios de KIRKBRIDE (1968) y BORHIDI (1985) reitero que el centro de evolución primario del género son Las Antillas Mayores y Cuba forma parte de ellas, pero no podemos obviar el aporte de Las Antillas Menores que al igual que Cuba tiene entre sus representantes especies que pertenecen a las secciones Odoratae, Rondeletia y Chamaebuxifoliae que son probablemente de las más antiguas del género, coincidiendo en las dos primeras una serie de caracteres morfológicos poco evolucionados, como se expuso con anterioridad, que hace difícil afirmar cuál es el centro de origen, cuál el centro de evolución primario del mismo, máxime sin que existan evidencias fósiles, de ahí que plantee las siguientes hipótesis:

— que el centro de evolución primario son Las Antillas Mayores y parte de América Central (en épocas pasadas) y que Cuba fue parte relevante del mismo;

— que a partir de Cuba, en períodos más recientes, las especies del género hayan emigrado hacía el resto de Las Antillas Menores, teniendo en cuenta que en Cuba están representadas especies que pertenecen a las secciones más antiguas (Rondeletia, Odoratae, Chamaebuxifoliae, etc.); emparentadas con especies que pertenecen a éstas secciones y que viven en otras islas de Las Antillas y Panamá. Además Odoratae y Chamaebuxifoliae, tienen gran flexibilidad ecológica, que puede interpretarse como signos de antigüedad, porque las especies más jóvenes están más especializadas y restringidas a ecótopos determinados, cosa que no se observa en los taxa posiblemente más antiguos, que presentan un área de distribución mayor y se adaptan a diferentes condiciones ecológicas, por lo que considero hayan sobrevivido, ejemplo R. odorata y R. chamaebuxifolia;

— que las especies encontraron mayor territorio, nichos ecológicos adecuados para establecerse y diversificarse en Cuba, teniendo en cuenta que en Las Antillas Menores y Panamá está representado por un número menor de especies, elevándose éste número hacia las islas mayores que están más cerca de Cuba (fig. 2), disminución que puede ser representada matemáticamente (fig. 1);

— otra interpretación sería considerar su evolución a partir de un centro continental, independientemente del número de especies, ya que los géneros más antiguos de la tribu y las secciones menos evolucionadas estuvieron allí representados y al emigrar hacía Las Antillas, el ambiente seco encontrado en algunas de las islas, estimuló la especiación, de ahí que las secciones menos evolucionadas estén mejor representadas en las islas de floras menos especializadas, donde el ambiente se parece más al clima conti-

nental (Jamaica y A. Menores) y las más evolucionadas se concentran en mayor número en Cuba y La Española, donde la variabilidad de ecótopos es mayor que en las demás.

Todas las secciones tienen representantes en caliza y serpentina, en diferentes tipos de vegetación y a diferentes alturas con relación al nivel del mar, lo que evidencia la riqueza del género; entre ellas los representantes de Lindenianae crecen en Cuba sobre serpentina, en La Española no; Chamaebuxifoliae tiene en Cuba taxa que viven en serpentina y caliza y su distribución es similar a la de algunos géneros que en la flora de Cuba presentan especies vicariantes en Cajálbana y el N de Oriente; las especies de ésta última región están bien definidas y especializadas, no así las de Cuba Occidental (R. chamaebuxifolia y R. longibracteata) lo que permite interpretar que las especies no estuvieron sometidas a iguales condiciones de aislamiento en éstas regiones.

Debemos destacar que las 10 secciones que conforman el género están presentes en Cuba, solo dos (Rondeletia y Chamaebuxifoliae) llegan hasta Las Antillas Menores y tres (Nipenses, Calophyllae, Leoninae) son exclusivas de Cuba; de ellas destaco Calophyllae porque está representada en el N de Oriente, Sierra Maestra y S. de Imías, donde sus taxa se sustituyen unos a otros geográficamente (Nipe: R. alaternoides ssp. alaternoides; Cristal: R. grandisepala y R. lucida; Moa: R. myrtacea ssp. brachyloba; Toa: R. galanensis; Imías: R. myrtacea ssp. myrtacea; S. Maestra: R. ekmanii, R. subglabra y R. calophylla).

JUDD (1981) plantea que una especiación extensiva y radiación adaptativa ha ocurrido en las Antillas Mayores (principalmente en Cuba y La Española), con especies que aparecen en un amplio rango de elevaciones, sobre diversos tipos de suelos y en varios tipos de vegetación, muchas son endémicos estrictos y otros tienen características morfológicas o anatómicas inusuales. Teniendo esto en cuenta a Rondeletia le son afines muchas de las propiedades anteriormente señaladas y aparentemente ha sufrido radiación adaptativa en cada isla, sobre todo en Cuba que sus especies no están presentes en otras islas del área.

Respecto a las secciones, todas están representadas en Cuba Oriental, cuatro en Cuba Central y cuatro en Cuba Occidental, lo que se corresponde con el número de especies representadas en estos sectores (SAMEK, 1973) o subprovincias (BORHIDI, 1985) fitogeográficas. Muchos taxa son propios de las provincias orientales o de Pinar del Río, aunque existen especies en las montañas de Villa Clara, cerca del centro de la Isla. Analizando éste tipo

de distribución dentro del archipiélago cubano tenemos que tener en cuenta que éste género está en proceso de evolución continua, cuyo centro de diversificación evidentemente le corresponde a Cuba Oriental, donde sus representantes más evolucionados están en mayor número; y se aprecia un proceso de especiación constante, confirmado porque muchos de los nuevos taxa son de ésta región fitogeográfica; Rondeletia es especialmente diverso en las montañas del N de Oriente, área que es también el centro de diversificación de muchos otros géneros. Las Sierras Maestra, de Nipe, Cristal, Moa, Toa y la región de Baracoa, muestran la mayor diversidad de especies del género y coinciden algunas localidades en estar aisladas geográficamente y en tener condiciones ecológicas extremas (las altas elevaciones, los pinares de la S. Maestra, los suelos lateríticos rojos de algunas áreas de Moa, Baracoa y Nipe), donde viven por ejemplo, R. intermixta ssp. intermixta y R. intermixta ssp. turquinensis. Las especies representadas en Jamaica y La Española viven en zonas montañosas elevadas y bajo distintas condiciones geográficas y ecológicas (JUDD, 1981), de ahí que cada montaña tenga sus especies características. En contraste con las especies de endemismo restringido que aparecen en Cuba, existen especies en el área de distribución del género, que muestran amplia variabilidad al presentar más de una variable geográfica, ejemplo R. inermis (Spreng.) Krug et Urb., especie muy polimorfa que presenta en Puerto Rico cinco variedades: var. inermis, var. angustifolia, var. intermedia, var. latifolia y var. oblongifolia, todas de Krug y Urb.

BORHIDI (1985) planteó que numerosas especies de la flora latifolia de Honduras tropical se establecieron temprano, se adaptaron al nuevo clima árido y algunos centros de evolución secundario se desarrollaron. Diferentes familias neotropicales y géneros representados por hojas membranáceas, macrófilas y mesófilas en el continente sufrieron un cambio tal, que grupos de especies o secciones enteras nuevas formadas por representantes con hojas micrófilas, esclerófilas y coriáceas aparecieron en Cuba y entre otras cita al género Rondeletia. De acuerdo con esto y a modo de ejemplo puedo citar la sección Hypoleucae que la conforman especies con caracteres que se corresponden con los anteriormente enunciados, por lo que es única en el área, es la más jóven y evolucionada del género y la que agrupa mayor número de especies, de ellas, la mayoría están en Cuba Central y Cuba Oriental, generalmente en la base de las montañas o en lugares muy secos (extremos), donde han sufrido una gran adaptación y diversificación debido a la variación ecológica y topográfica de la zona, mientras en otras secciones no se ob-

serva éste fenómeno. El grado de variación morfológica se corresponde con éste análisis, en Hypoleucae la variación es discontinua, permitiendo el reconocimiento de muchas especies, mientras en otras secciones como Calophyllae la variación tiende a ser contínua, lo que se manifiesta en el sentido amplio con que se interpretaron algunos taxa por botánicos anteriores, ejemplo el "complejo alaternoides" donde fué posible reconocer un gradiente de caracteres diferentes en las especies que forman parte de ésta sección y que viven en las diferentes montañas de la región oriental y la selección de algunas variedades geográficas dentro del grupo.

Además existen ecótopos de la isla y del territorio pinero que el género aún no ha invadido, por ejemplo ninguno de sus representantes crece en arenas blancas, lo que coincide con la hipótesis de López Almirall (com. personal); que plantea que Isla de La Juventud actúa como refugio de taxa más antiguos y no se hallan los grupos más jóvenes desde el punto de vista evolutivo, hipótesis que se fundamenta en la teoría de THORNE (1969) que señala que las Islas Continentales, como muchos archipiélagos aislados (específicamente las Islas Californianas) funcionan primariamente como refugios de relictos, más que de elementos evolucionados. Por otra parte, se debe tener en cuenta que Rondeletia, aparentemente, no prefiere ecótopos arenosos, teniendo en cuenta que una de las especies más antiguas de Cuba Occidental (R. odorata), no se ha refugiado en I. de La Juventud, aunque ésta planta tiene una ecología amplia, en lo que a preferencia del suelo se refiere. Es de suponer, también, que la ausencia de Rondeletia en el territorio pinero, se deba a la pobreza de la zona en ecótopos extremadamente secos como: serpentina y áreas cársicas que aparentemente favorecen la especiación de éste taxon.

El género presenta una amplia variedad de habitats, pero prefiere suelos serpentínicos (serpentina esquelética, ferrítico púrpura, fersialítico pardo rojizo); de calizas (esquelético rendzina roja y negra, dolomitas, carso en complejo de vegetación de mogote, etc.) y suelos muy diversos presentes en las diferentes formaciones arbóreas, atendiendo a la altitud y a la roca madre.

Entre las especies que crecen en suelo serpentínico podemos citar a R. venosa, R. odorata y R. chamaebuxifolia en Cajálbana; R. acunae, R. plicatula, R. bissei, R. toaensis, R. miraflorensis, R. savannarum, R. steirophylla, R. lucida, R. cristalensis, R. diplocalyx, R. paucinervis, R. nipensis, R. pycnophylla, R. grandisepala, R. alaternoides, R. lomensis, R. galanensis, R. glomeruliflora en el Norte de Oriente; R. camarioca, R. rugelii,

R. odorata en Habana-Matanzas; R. camarioca, R. odorata en las serpentinas de Cuba Central. La evolución de los caracteres morfológicos en general no se corresponden exactamente con la evolución de la serpentina, porque en todos los núcleos de serpentina independientemente de su antiguedad están representadas especies de las secciones más o menos evolucionadas, por ejemplo Hypoleucae, tiene representantes en todas las áreas de serpentina del país, destacándose en el Norte de Oriente, con un mayor número de especies, los que exhiben diferentes caracteres evolucionados que permiten la adaptación de esos taxa a condiciones extremas, de tal forma que la mayoría están restringidas a areales puntiformes (R. bissei, R. miraflorensis, R. steirophylla). Por otro lado, Odoratae, a pesar de tener caracteres poco evolucionados en mayor número no está presente en el N de Oriente, que es el núcleo más antiguo para la serpentina, sin embargo en Cajálbana está presente R. odorata ssp. bullata que es el taxon más evolucionado del grupo y las otras dos subespecies están en el resto de las áreas de serpentina al igual que en caliza, sin preferencia alguna, lo que evidencia su flexibilidad ecológica y sus posibilidades de adaptación.

Entre las especies que crecen en caliza o en suelos derivado de ella tenemos R. chamaebuxifolia, R. peninsularis, R. odorata ssp. odorata y ssp. grandifolia, R. pedicellaris, R. hypoleuca, R. microphylla, R. linearisepala, R. norlindii, R. combsii, R. susannae.

Todos estos taxa exhiben entre ellos vicarianza geográfica, ecológica o cenológica, sustituyéndose unos a otros en diferentes localidades tal como se expuso en el epígrafe 3.2.3. al citar algunos ejemplos.

Las especies cubanas están presentes en distintas formaciones vegetales a diferentes altitudes (desde el nivel del mar hasta aproximadamente 1700 m.s.n.m.), entre ellas tenemos el matorral xeromorfo costero y subcostero, matorral xeromorfo espinoso sobre serpentina, matorral xeromorfo sub-espinoso sobre serpentina, complejo de vegetación de mogote, bosque de pinos, bosque pluvial montano, bosque nublado, bosque siempreverde mesófilo, bosque siempreverde micrófilo, bosque de galería.

3.4. Estado de conservación de las especies

Por último, abordaré un tema que considero un buen resultado producto de ésta investigación y está relacionado con el estado de conservación actual de las especies, ya que la mayoría crecen en formaciones vegetales típicas y naturales que cada díe se ven en peligro a consecuencia del incre-

mento de la actividad humana y ello trae aparejado la urbanización de campos y montañas, por lo que exhorto a una atención especial a las zonas de alto endemismo de las especies de Rondeletia, que en su mayoría coinciden con áreas de interés botánico para otros taxa de la flora de Cuba; destaco que el estado de conservación de algunas especies las ubica en las categorías definídas en el Libro Rojo de Datos de la UICN (1981). Entre ellas están las extintas (Ex) como R. azulensis Urb., R. potrerillona Urb. y R. intermixta Britt. ssp. turquinensis Fernández et Borhidi, R. alaternoides A. Rich. ssp. alaternoides, de las que sólo conocemos la colección original y los esfuerzos por relocalizarlas en su locus clásico han sido en vano; otro grupo de especies manifiesta una notable reducción de sus individuos en las poblaciones, por lo que se consideran en peligro de extinción (E) ejemplo R. rugelii Urb., R. monantha Urb. et Ekm., R. apiculata Urb., R. leonis Britt., R. acunae Borhidi et Fernández, R. convoluta Fernández et Borhidi y R. subglabra Krug et Urb.; se consideran vulnerables (V) R. subcanescens Fernández et Borhidi, R. cristalensis Urb., R. baracoensis Britt., R. insularis Britt., R. miraflorensis Fernández et Borhidi y R. venosa Wr. et Griseb.; por último categorizadas como raras (R), teniendo en cuenta el riesgo que corren, dada la posición geográfica que ocupan y la restricción de sus habitats están R. bicolor Britt., R. diplocalyx Urb., R. ekmanii Britt., R. micarensis Urb., R. paucinervis Urb., R. pycnophylla Urb. Son taxa fundamentalmente de areales restringidos y ésto incrementa la necesidad de su conservación; no obstante, no obviamos que éste fenómeno de vulnerabilidad caracteriza en la actualidad a las floras insulares, de ahí que hoy sea una solicitud general el cuidado, atención y protección de todos los taxa y áreas de interés botánico de todo el mundo, reclamo vinculado al interés de utilizar los recursos naturales en beneficio de toda la humanidad.

Clave para las especies cubanas del género Rondeletia

1 a Hojas con un tomento blanco o amarillento apretado en el envés, por lo menos cuando jovenes 37
  b Hojas sin tomento en el envés, a veces con pelos estrigilosos en los nervios 2
2 a Flores pediceladas 3
  b Flores sentadas 21
3 a Inflorescencia corimbosa, terminal .... 1. R. odorata

b Inflorescencia cimoso-paniculada, cimoso-racemosa o uniflora, mayormente axilar 4
4 a Inflorescencia cimoso-paniculada o cimoso-racemosa 5
b Inflorescencia uniflora o cimoso-3-flora 12
5 a Inflorescencia cimoso-racemosa axilar, hojas aovadas u oblongo-aovadas, mates en ambas caras .... 2. R. intermixta ssp. turquinesis
b Inflorescencias cimoso-paniculadas 6
6 a Hojas acuminadas y agudas en el ápice .... 3. R. calophylla
b Hojas redondeadas u obtusas en el ápice 7
7 a Lóbulos del cáliz espatulados 8
b Lóbulos del cáliz lineales 10
8 a Inflorescencia pubérula o hirsuta 9
b Inflorescencia glabra .... 4. R. lucida
9 a Lóbulos del cáliz obovado-espatulados, cortos de 1-1,5 mm, inflorescencia densamente corto-hirsuta .... 5. R. alaternoides
b Lóbulos del cáliz oblongo-espatulados de 2-3 mm, gruesos, inflorescencia apretado-pubérula .... 6. R. galanensis
10 a Inflorescencia glabrescente con pocos pelos apretados, lóbulos del cáliz lineales o lineal-espatulados de 1,5-2 mm de largo; hojas oblongo-elípticas .... 7. R. subglabra
b Inflorescencia densamente albo-pelosa o pubescente 11
11 a Lóbulos del cáliz subulados de 1 mm de largo; hojas suborbiculares a elípticas .... 8. R. ekmanii
b Lóbulos del cáliz lineales y obtusos, ligeramente ensanchados en el ápice, muy cortos; hojas elípticas u oblongo-elípticas .... 9. R. myrtacea ssp. brachyloba
12 a Inflorescencias o flores terminales 13
b Inflorescencias o flores axilares o laterales 19
13 a Hojas pergamáceas a cartáceas, mates por lo menos en el envés 14
b Hojas coriáceas, brillantes en ambas caras 16
14 a Hojas escabroso-pelosas en el haz, pedicelos muy delgados y flexuosos, flores 4-meras .... 10. R. pedicellaris
b Hojas lampiñas en el haz, pelosas sólo en el nervio medio; flores 5-meras 15
15 a Tubo del cáliz glabro .... 11. R. peduncularis
b Tubo del cáliz pubescente .... 12. R. microphylla
16 a Hojas de 2-3 mm de largo, elípticas .... 13. R. minutifolia
b Hojas más grandes 17

17 a Hojas muy revolutas, más largas que las inflorescencias .... 14. R. pycnophylla

b Hojas de margen recurvo, mucho más cortas que el pedúnculo 18

18 a Tubo del cáliz peloso .... 15. R. shaferi

b Tubo del cáliz glabro .... 16. R. pachyphylla

aa Hojas de 2-3 cm de largo, aovadas a elípticas, nervios no reticulados en el haz .... 16.a. ssp. pachyphylla

bb Hojas de 1-2 cm de largo, obovadas, nervios prominulos y reticulados en el haz .... 16.b. ssp. myrtilloides

19 a Hojas mayormente en vertícilos de 4, cartáceas, mates en ambas caras .. .. 17. R. lindeniana

b Hojas opuestas, coriáceas, brillantes por lo menos en el haz 20

20 a Estípulas triangulares, obtusas, hojas no domaciadas, pedúnculo de 7-8 cm de largo .... 18. R. nima-nimae

b Estípulas lanceolado-subuladas, mucronadas, hojas a menudo domaciadas, pedúnculo de 1-5 cm de largo .... 19. R. leonis

c Estípulas triangulares, lanceo-acuminadas, hojas glabras o pelositas en los nervios por el envés, pedúnculo de 10-15 mm de largo .... 20. R. monantha

21 a Hojas brillantes en ambas caras 22

b Hojas mates en ambas caras 24

22 a Flores en capítulos involucrados, involucro ferruginoso-peloso .... 21. R. rigida

b Flores en cabezuelas pedunculadas, no involucradas 23

23 a Inflorescencia densamente albo-pubescente; ramitas albo-estrigosas, pedúnculo de 2-4 cm de largo, lóbulos del cáliz oblanceolados de 3,5-4 mm de largo .... 22. R. naguensis

b Inflorescencia ferrugíneo-pelosa, pedúnculos de hasta 1,5 cm de largo, lóbulos del cáliz orbicular-espatulados de 1,5-2 mm de largo .... 23. R. grandisepala

24 a Inflorescencias en cabezuelas axilares o terminales en las ramitas laterales 25

b Inflorescencias unifloras o flores solitarias axilares 27

25 a Capítulos sin brácteas connadas en la base, hojas plateado-seríceas cuando jóvenes, flores mayormente 4-meras .... 24. R. nipensis

b Capítulo subtendido por brácteas involucrales, flores mayormente 5-meras 26

26 a Flores rosadas, hojas aovadas de hasta 1,5 cm de largo . 25. R. vazquezii

b Flores blancas, hojas más grandes .... 26. R. lomensis

27 a Flores subtendidas por bracteolas connadas formando un tubo caliciforme encerrando el cáliz 28

b Bracteolas libres o algo connadas, no formando un tubo caliciforme . 30

28 a Bracteolas involucrales lanceoladas de 5-9 mm de largo, cápsula de 5-7 mm de diámetro, hojas coriáceas .... 27. R. bracteosa

b Brácteas involucrales de hasta 3 mm de largo 29

29 a Hojas de 1,5-2,5 cm, aovadas, agudas y apiculadas en el ápice, brácteas involucrales de hasta 3 mm de largo .... 28. R. diplocalyx

b Hojas de 6-10 mm, obovadas, redondeadas en el ápice, brácteas involucrales de hasta 1,5 mm de largo .... 29. R. toaensis

30 a Hojas de 3-15 mm de largo 31

b Hojas de más de 15 mm de largo 34

31 a Flores 5-meras, hojas oblongo-lanceoladas u oblongo-elípticas, lóbulos del cáliz lineales .... 30. R. vaccinifolia

b Flores 4-meras, hojas aovadas u obovadas 32

32 a Hojas estrigiloso-pelosas en ambas caras cuando jóvenes, reticulación de los nervios muy prominente en el envés, lóbulos del cáliz redondeados .... 31. R. arida

b Hojas glabras en el haz, reticulación no muy prominente en el envés, lóbulos del cáliz agudos a obtusos 33

33 a Hojas de 3-8 mm, obovadas, obtusas o agudas en la base .... 32. R. paucinervis

b Hojas de 8-13 mm, aovadas a orbiculares, redondeadas a truncadas en la base .... 33. R. cristalensis

34 a Lóbulos del cáliz triangulares a aovados de 1-2 mm de largo 35

b Lóbulos del cáliz lineares, de 2-2.5 mm, brácteas lineares .... 34. R. avenia

35 a Hojas obovadas a oblongo-obovadas, redondeadas a muy obtusas en el ápice, el envés reticulado-estrigiloso .... 35. R. insularis

b Hojas elípticas o aovadas, estrechadas a agudas en el ápice, el envés no reticulado venoso, glabro 36

36 a Hojas mayormente elípticas, atenuadas y agudas en ambos extremos, glabras en ambas caras; lóbulos del cáliz triangulares o aovados, de 1 mm .... 36. R. chamaebuxifolia

b Hojas aovadas, redondeadas a truncadas en la base, pubérulas en el nervio medio del envés, lóbulos del cáliz lanceolados de 2-2.5 mm .... 37. R. micarensis

37 a Hojas lampiñas en el haz, aún cuando jóvenes, o algo pubérulos a lo largo del nervio medio 38

b Hojas fina y densamente pubescentes en el haz cuando jóvenes, la pubescencia comunmente persistente 57

38 a Hojas glabrescentes a glabras en el envés, con la edad, flores en cabezuelas pedunculadas 39

b Hojas con un tomento persistente en el envés, flores no en cabezuelas. 40

39 a Hojas redondeadas a muy obtusas en el ápice, lóbulos del cáliz 4, triangulares, connados en la base .... 26. R. lomensis

b Hojas agudas a apiculadas en el ápice, lóbulos del cáliz 4-5, aovado-deltoideos, de 1,5-2 mm .... 24. R. nipensis

40 a Inflorescencia cimoso-racemosa, 5-11-flora .... 2.b. R. intermixta ssp. intermixta

b Inflorescencia 1-3-flora 41

41 a Hojas oblongo-elípticas a oblongo-ovales de 2-4.5 cm de largo, el haz negro cuando seco, el envés muy plateado 42

b Hojas de otras formas y de menor tamaño 43

42 a Flores con pedicelos de 3 mm, lóbulos del cáliz lineales, de 3-4 mm; hojas de 2-3 cm de largo .... 38. R. bicolor

b Flores con pedicelos de 4-10 mm de largo, lóbulos del cáliz subulados de 4-5 mm; hojas de 3-5 cm .... 39. R. susannae

43 a Lóbulos del cáliz aovado-deltoideos a suborbiculares de 0.5-2 mm de largo 44

b Lóbulos del cáliz lineales o lineal-subulados de 2-7 mm de largo ... 48

44 a Hojas de 1-2,5 cm de largo 45

b Hojas de hasta 1-1,5 cm de largo 47

45 a Flores subsésiles con brácteas connadas formando un tubo caliciforme, lóbulos del cáliz connados en tubo con dientes apiculados muy cortos de 0.2-0.5 mm .... 40. R. tubulosa

b Brácteas no connadas en un tubo caliciforme 46

46 a Ramitas antrorso-estrigilosas, hojas con nervios laterales no encorvados hacía el ápice, muy poco reticulados, flores pedunculadas, 4-meras .... 41. R. subcanescens

b Ramitas retrorso-pelosas, flores sentadas, 5-meras, hojas con una reticulación densa, nervios laterales encorvados hacía el ápice .... 42. R. baracoensis

47 a Ramitas retrorso-estrigilosas, reticulación de los nervios no aparente en el envés .... 43. R. bissei

b Ramitas antrorso-estrigilosas, reticulación muy aparente .... 44. R. apiculata

48 a Cápsula de 6-10 mm, lóbulos del cáliz de 3-7 mm o más, connados en la base, brácteas connadas .... 45. R. coronata

b Cápsula y lóbulos del cáliz más pequeños 49

49 a Hojas lanceoladas a elípticas, de más de 1.5 cm, agudas en el ápice, el margen muy revoluto, lóbulos del cáliz mayormente subulados aguados . 50

b Hojas orbiculares u orbicular-aovadas u obovadas, mayormente menos de 1.5 cm, lóbulos del cáliz lineales, obtusos 52

50 a Flores 4-meras .... 46. R. venosa

b Flores 5-meras 51

51 a Hojas mucronadas en el ápice, lóbulos del cáliz muy desiguales, lóbulos de la corola glabros por dentro, cápsula de hasta 2 mm .. 47. R. acunae

b Hojas no mucronadas, muy revolutas, lóbulos del cáliz iguales, reflejos, connados en la base, lóbulos de la corola tomentosas por dentro .... 48. R. convoluta

52 a Hojas obovadas, cuneadas en la base, pelos del nervio medio antrorsos 53

b Hojas orbiculares u aovadas, obtusas a redondeadas en la base 54

53 a Hojas de 8-15 mm, lóbulos del cáliz lineal-subulados de 2.5-3 mm, cápsula de 4-5 mm .... 49. R. savannarum

b Hojas de 6-10 mm, lóbulos del cáliz lineales, de 4-5 mm, obtusos en el ápice, cápsula de 3-4 mm .... 50. R. plicatula

54 a Hojas orbiculares a suborbiculares, el margen revoluto, nervios laterales hundidos en el haz .... 51. R. hypoleuca

b Hojas aovadas a elíptico-aovadas u obovadas, apiculadas en el ápice, el margen plano, nervios laterales nulos en el haz 55

55 a Hojas con nervios muy ensanchados en la base del envés, brácteas espatulado-deltoideas, redondeadas en el ápice 56

b Nervio medio no muy ensanchado en la base, por el envés, brácteas triangulares a lineal-aovadas, atenuadas en el ápice . 52. R. miraflorensis

56 a Pelos en el nervio medio del envés antrorsos, brácteas orbicular-espatuladas .... 53. R. steirophylloides

b Pelos en el nervio medio del envés retrorsos, brácteas oblongo-espatuladas .... 54. R. steirophylla

57 a Hojas oblongo-lanceoladas a oblongo-elípticas, agudas o ligeramente estrechadas en ambos extremos 58

b Hojas aovadas u obovadas, no agudas o acuminadas en ambos extremos . 63

58 a Hojas de 1.5-5 cm de largo 59

b Hojas de hasta 2 cm de largo 61

59 a Hojas a menudo glabrescentes con la edad, ramitas antrorso-pelosas . 60

b Hojas retrorso-pelosas, tomento de las hojas persistentes .... 55. R. papayoensis

60 a Lóbulos del cáliz lineal-lanceolados, agudos, de 2-3 mm de largo .... 56. R. combsii

b Lóbulos del cáliz triangulares, cortos, de 1-1.5 mm de largo, connados en la base, reflejos .... 57. R. combsioides

61 a Hojas glabras con la edad en el haz, negras al secar, flores solitarias, lóbulos del cáliz filiformes, acuminados de 2 mm de largo .... 58. R. potrerillona

b Hojas con pubescencia persistente en el haz, hojas no negruzcas al secar, lóbulos del cáliz más cortos y no acuminados 62

62 a Hojas con peciolos de 1-2 mm, lóbulos del cáliz lineales, de 1-1.5 mm .... 59. R. rugelii

b Hojas con pecíolos de 2-6 mm; lóbulos del cáliz triangulares, de hasta 1 mm .... 60. R. ingrata

63 a Ramitas retrorso-pelosas 64

b Ramitas antrorso-pelosas 66

64 a Flores 5-meras .... 61. R. linearisepala

b Flores 4-meras 65

65 a Lóbulos del cáliz aovados a triangulares .... 62. R. peninsularis

b Lóbulos del cáliz lineales .... 63. R. azulensis

66 a Hojas de hasta 1.5 cm de largo, elípticas, aovadas a lanceoladas, mayormente agudas a apiculadas en la base .... 44. R. apiculata

b Hojas obovadas mayormente más grandes, redondeadas en el ápice, estrechadas en la base, bracteas libres .... 64. R. camarioca

4. Conclusiones

1. Rondeletia correifolia, de Cuba Occidental, pertenece a un nuevo género para la ciencia: Roigella, endémico y monotípico.
2. Las especies que incluían en su sinonimia al género Ferdinandusa, pertenecen a Suberanthus, género de Cuba y La Española.
3. Se describieron, 21 nuevos taxa para la ciencia del género Rondeletia L. s.s. (17 especies y cuatro subespecies). Cinco especies pasaron a la sinonimia de otras y se excluyeron nueve del género.

4. El estudio del género Rondeletia L. s.s., evidenció la existencia de 65 especies en Cuba, todas endémicas.
5. Se propusieron 10 secciones nuevas (todas representadas en Cuba), para la comprensión sistemática del género.
6. Se confeccionaron las claves dicotómicas correspondientes para los géneros incluídos en Rondeletieae y sus aliados; para la secciones de Rondeletia y para las especies que incluye.
7. Se obtuvieron tres tipos principales de granos de polen: Tipo Rondeletia, Tipo Roigella y Tipo Suberanthus.
8. Se delimitó la distribución geográfica del género y los límites o extensión de cada una de las secciones.
9. El coeficiente de similitud que se aplicó para correlacionar los géneros (Roigella, Rondeletia y Suberanthus) y las secciones, corroboraron los resultados cótenidos desde el punto de vista taxonómico. Con relación a las secciones, se evidenció que existen dos grupos básicos dentro de Rondeletia. El análisis de componentes principales (ACP) aplicado puso de manifiesto la identidad de las secciones.
10. El origen de Rondeletia se explica a partir de la existencia de un centro de volución primario en Las Antillas Mayores y parte de América Central (Panamá), donde Cuba formó parte relevante del mismo, en épocas antiguas (analizando número de especies); o de un centro de evolución primario en la parte N de América del Sur incluída Panamá, considerando a Cuba como parte relevante del centro de evolución secundario, representado por Las Antillas, durante el Terciario (analizando la antiguedad).
11. En Cuba Oriental, donde se localizan el mayor número de especies, la mayor diversidad y la mayoría de los taxa más evolucionados del grupo, hay un centro de diversificación.
12. Se comprobó que Rondeletia aparece sobre diversos tipos de suelo, a diferentes alturas y en casi todos los tipos de vegetación. Muchas de sus especies son endémicas estrictas.
13. Se pudo corroborar el estado crítico de Rondeletia con relación a su conservación. Este cuenta con cuatro especies extintas (Ex), siete en peligro de extinción (E), seis raras (R) y seis vulnerables (V), aspecto que pone de manifiesto la crisis poblacional de las especies cubanas.

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STUDIES IN RONDELETIEAE, XII. NEW COMBINATIONS OF MEXICAN AND CENTRAL AMERICAN TAXA

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New combinations of 16 Mexican and Central American species of Rondeletia s.l. are proposed classifying 15 of them to the genus Arachnothryx Planch. and 1 to the genus Rogiera Planch.

Introduction

A remarkable paper was published by David H. LORENCE (1991), where he described 13 new Rondeletia species from Mexico and Central America. As he pointed out, he wanted to maintain the genus Rondeletia in a broad sense according to HEMSLEY (1879) and HOOKER (1873), although he had not any difficulties to recognize the characters of Arachnothryx and Rogiera on his material.

My opinion, explained earlier in several papers (BORHIDI 1982, 1989; BORHIDI and FERNANDEZ 1981a, b), that the generic concept of Rondeletia has to turn back to the original Linnean sense as it was firstly proposed by STEYERMARK (1967) who revalidated the genus Arachnothryx and excluded Rondeletia schumanniana Krause (a form of Rogiera amoena) from the genus Rondeletia.

For a more detailed critical discussion and argumentation of this question, see the article in the next volume.

According to our generic concept the following new combinations are proposed:

Arachnothryx affinis (Hemsl.) Borhidi **comb. nova** — Mexico

Basionym: Rondeletia affinis Hemsley Diagn. Pl. Nov. Mexic. 28. 1879.

Arachnothryx atravesadensis (Lorence) Borhidi **comb. nova** — Mexico

Basionym: Rondeletia atraveşadensis Lorence in Novon, 1: 137. 1991.

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Arachnothryx chaconis (Lorence Borhidi **comb. nova** — Costa Rica

Basionym: Rondeletia chaconis Lorence in Fieldiana Bot. Nov. Ser. 33: 296. 1993.

Arachnothryx dwyeri (Lorence) Borhidi **comb. nova** — Panama

Basionym: Rondeletia dwyeri Lorence in Novon, 1: 139. 1991.

Arachnothryx ginetteae (Lorence) Borhidi **comb. nova** — Mexico

Basionym: Rondeletia ginetteae Lorence in Novon, 1: 141. 1991.

Arachnothryx guerrerensis (Lorence) Borhidi **comb. nova** — Mexico

Basionym: Rondeletia guerrerensis Lorence in Novon, 1: 143. 1991.

Arachnothryx manantiensis (Lorence) Borhidi **comb. nova** — Mexico

Basionym: Rondeletia manantiensis Lorence in Novon, 1: 145. 1991.

Arachnothryx monteverdensis (Lorence) Borhidi **comb. nova** — Costa Rica

Basionym: Rondeletia monteverdensis Lorence in Novon, 1: 147. 1991.

Arachnothryx povedae (Lorence) Borhidi **comb. nova** — Costa Rica

Basionym: Rondeletia povedae Lorence in Fieldiana Bot. Nov. ser. 33: 299. 1993.

Arachnothryx purpurea (Lorence) Borhidi **comb. nova** — Mexico

Basionym: Rondeletia purpurea Lorence in Novon, 1: 148. 1991.

Arachnotrhyx ricoi (Lorence) Borhidi **comb. nova** — Mexico

Basionym: Rondeletia ricoi Lorence in Novon, 1: 152. 1991.

Arachnothryx rzedowskii (Lorence) Borhidi **comb. nova** — Mexico

Basionym: Rondeletia rzedowskii Lorence in Novon, 1: 154. 1991.

Arachnothryx scoti (Lorence) Borhidi **comb. nova** — Mexico

Basionym: Rondeletia scoti Lorence in Novon, 1: 155. 1991.

Arachnothryx taylorae (Lorence) Borhidi **comb. nova** — Costa Rica

Basionym: Rondeletia tayloriae Lorence in Fieldiana Bot. Nov. Ser. 33: 300. 1993.

Arachnothryx tenorioi (Lorence) Borhidi **comb. nova** — Mexico

Basionym: Rondeletia tenorioi Lorence in Novon, 1: 156. 1991.

Rogiera macdougallii (Lorence) Borhidi **comb. nova** — Mexico

Basionym: Rondeletia macdougallii Lorence in Novon, 1: 144. 1991.

Because of a printing error some new combinations published in the Acta Botanica Hungarica 35: 311 are not completely readable. Therefore they are here repeated:

Arachnothryx rubens (L.O.Wms.) Borhidi **comb. nova**

Basionym: Rondeletia rubens L.W. Wms., in Phytologia 28 (2): 128. 1973.

Arachnothryx silvicola (L.O.Wms.) Borhidi **comb. nova**

Basionym: Rondeletia rubens L.O.Wms., in Phytologia 28 (2): 128. 1973.

Arachnothryx tuxtlensis (Lorence & Castillo-Campos) Borhidi **comb. nova**

Basionym: Rondeletia tuxtlensis Lorence & Castillo-Campos in Biotica 13: 148. 1988.

Arachnothryx urophylla (L.O.Wms.) Borhidi **comb. nova**

Basionym: Rondeletia urophylla L.O.Wms. in Phytologia 28 (2): 129. 1973.

Arachnothryx uxpanapensis (Lorence & Castillo-Campos) Borhidi **comb. nova** — Mexico

Basionym: Rondeletia uxpanapensis Lorence & Castillo-Campos in Biotica 13: 150. 1988

Arachnothryx wendtii (Lorence & Castillo-Campos) Borhidi **comb. nova** — Mexico

Basionym: Rondeletia wendtii Lorence & Castillo-Campos in Biotica, 13: 154. 1988.

Arachnothryx yucatanensis (Lundell) Borhidi **comb. nova** — Mexico

Basionym: Rondeletia yucatanensis Lundell in Wrightia, 5 (8): 329. 1976.

Rogiera seleriana (Loesener) Borhidi **comb. nova**

Basionym: Rondeletia seleriana Loesener in Verh. Bot. Verin. Brandbg., 65: 105. 1913.

Rogiera subscandens (Lundell) Borhidi **comb. nova** — Mexico

Basionym: Rondeletia subscandens Lundell in Wrightia 5 (8): 328. 1973.

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NUEVAS ESPECIES Y REVISIÓN BREVE DEL GÉNERO STENOSTOMUM C.F. GAERTIN. (RUBIACEAE) EN CUBA

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Six new species belonging to the revalidated genus Stenostomum are described: S. baracoense, S. biflorum, S. cuspidatum, S. imbricatum, S. reticulatum and S. revolutum. All the taxa described here are found in the oriental montane ranges of Cuba, one occurs in the Sierra Maestra, other in the Sierra de Cristal, two in Moa, and two in the serpentine ranges of Baracoa. Probably all the new taxa are endemic to Cuba. The authors included the genus Terebraria (Neolaugeria) into Stenostomum maintaining the original concept of GRISEBACH (1861) and BRITTON (1929). Together with all these new taxa the genus Stenostomum is actually represented by 27 species instead of the 19 ones listed in the Flora of Cuba (ALAIN 1962). The descriptions of the new taxa are followed by a new analytical key for the identification of the Cuban species and by a short review of them.

Introducción

Seis espécies nuevas pertenecientes al género revalidado Stenostomum C.F.Gaertn. (Rubiaceae) estan descritas, S. baracoense, S. biflorum, S. cuspidatum, S. imbricatum, S. reticuladum y S. revolutum. Todas fueron colectadas en las montañas de Cuba Oriental, una en la Sierra de Cristal, otra en la Sierra Maestra, dos en la region de Moa y dos en las serpentinitas de Baracoa. Probablemente todas son endémicas cubanas. Los autores incluyeron el género Terebraria (Neolaugeria) en el género Stenostomum manteniendo el concepto original de GRISEBACH (1861) y de BRITTON (1929). Con este articulo complementario, actualmente la flora del Cuba tiene 27 espécies de Stenostomum 8 más de lo que esta reportado en la Flora de Cuba de ALAIN (1962).

Después de las descripciones se presenta una clave analítica de identificación para determinar las espécies cubanas, y una lista anotada de estas espécies.

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Descripciones de los nuevos taxa

Stenostomum baracoense Borhidi sp. nova

(Antirhea baracoensis Borhidi in schaedis)

Arbor parva. Rami veteriores cinereo-nigrescentes, cicatricibus transverse fissurati, cylindracei, hornotini 4-anguli vel compressi et 2-alati, adresse pilosi vel glabri. Stipulae semiorbiculares, quam ramuli latiores, horizontaliter patentes, inter sese saepe breviter connatae, 1,5--2 mm longae et 4--6 mm latae, apice rotundatae vel brevissime apiculatae, dorso adpresse pilosae, margine ciliatae, coriaceae, lingescentes et persistentes. Yema terminalis internodiisque superioribus abbunde resinosa. Folia subsessilia, petiolis 1--2 mm longis, pilosis suffulta, elliptica, oblongo-elliptica vel obovata, 3—9 cm longa, 1—3,5 cm lata, nervo medio supra impresso, subtus prominente, lateralibus utroque latere 7--10 cum variis interjectis supra obsoletis, subtus paullo prominulis et reticulatis, ante marginem conjunctis, margine paullo recurva, subcoriacea vel coriacea.

Inflorescentiae uniflorae, in axillis foliorum superiorum sessiles. Calyx 5—6 mm longa, tubus ovarii 5-angulatus, 2—2,5 mm longus, limbus tubi liber cylindraceus, superne ampliatus, 2,5--3 mm longus, glaber, lobi 5, inaequales, 1,5—2 mm longi, semiorbiculares vel late ovatae, apice rotundatae vel obtusae, glabrae. Corolla non visa. Ovarium 2 mm longum, biloculare, ovula solitaria. Fructus oblongo-obovatus vel lineari-obovatus, 10--13 mm longus, angulatus, apice tubo calycino persistente 3--4 mm longe coronatus, glaber. Locula fructus aborto semen unum, 5--8 mm longum lineari-oblongum, embryo oblongus.

Holotypus: BISSE et al. 36878 HAJB; Cuba, Prov. Guantánamo, Baracoa, Quibiján, zona del Arroyo Blanco en camino a Vega de la Palma. 16.02.1978.

Specimina examinata: HAJB 4979, Baracoa, pluviosilva al Sur de Yunque de Baracoa, col.: BISSE & KÖHLER — HAJB 37151, Baracoa, orillas del Rio Duaba, cerca de Vega de la Palma, col.: BISSE et al. — HAJB 5196, Baracoa, valle al Noreste de Yunque de Baracoa, col.: BISSE & KÖHLER — ALAIN 7278 HAC. Baracoa, charrascal junto a la Via Mulata. Col.: ALAIN & LOPEZ FIGUEIRAS.

Obs.: Stenostomo abbreviato (Urb.) Borhidi & Fernandez affinis sed ab ea foliis ellipticis vel oblongis, basi rotundatis, multo majoribus, inflorescentiis unifloris, fructibus forma et magnitudine diversis clare distinguitur.

Stenostomum biflorum Borhidi sp. nova

(Antirhea biflora Borhidi in schaedis)

Frutex parvus. Rami veteriores cinereo-nigrescentes, longitudinaliter sulcato-striati, hornotini 4-anguli, minute pilosi et punctis emergentibus resinosis scabriusculi, apice ramorum dense foliigeri et resinam exsudantes, internodiis 1—2 cm longis, apicem versus 2—4 mm longis. Stipulae interpetiolares dorso bicarinatae, late ovatae, 2 mm longae, apice rotundatae, glabrae resinosae, deciduae. Folia subsessilia, 1—1,5 mm longe petiolata, oblongo elliptica, apice rotundata, basi obtusa vel rotundata, 1—1,8 cm longa et 0,4—0,7 cm lata, nervo medio supra valde impresso, subtus in sulco prominente, lateralibus 4—6 supra nullis, subtus vix conspicuis, obscure reticulatis, lamina supra nitida, ad marginem brevissime et parce pilosa, subtus glabra, margine revoluta et crasse coriacea.

Inflorescentiae ex axilla foliorum summorum sub apice ramorum abeuntes, abbreviatae, 2-florae. Pedunculus 3—5 mm longus, apice dichotomus, ramis 1—2 mm longis, prophylla nulla. Calyx totus 4—5 mm longus, tubus obovatus, limbo libero ovario aequilongo, superne ampliato, leviter bilabiato, breviter piloso. Lobi 5, adaxiales 3 longiores, ovati, limbo libero aequilongi, ovario duplo longiores, abaxiales 2 breves, semiorbiculares vel truncatae. Corolla alba, tubus cylindraceus, 4—5 mm longus, basi 2 mm crassus, intus glaber, lobi 5, imbricati, orbiculari-ovati, 1,5—2 mm longi et lati, tubo 2-plo breviores. Stamina 5, fauce affixa, inclusa, filamenta 0,5—1 mm longa, applanata, margine ciliata, antherae oblongo-ovatae, apice acutae, 2 mm longae. Stylus 3 mm longus, superne incrassatus glaber, apice brevitter bilobus. Ovarium 1-loculare, ovulum solitarium.

Holotypus: BISSE & KÖHLER 7091 HAJB. Cuba. Prov. Santiago de Cuba, Sierra del Cristal, Charrascal de Saca La Lengua, 600 m.s.m.

Obs.: Stenostomo abbreviato (Urb.) Borhidi & Fernandez affinis, a qua foliis oblongo ellipticis, valde coriaceis, nervis lateralibus supra nullis subtus vix conspicuis, tubo corollino crassiore et duplo breviore atque staminibus filamentosis, ovario uniloculare clare differt.

Stenostomum cuspidatum Borhidi sp. nova

(Neolaugeria cuspidata Borhidi in schaedis)

Frutex vel arbor parva usque add 4—5 m alta. Rami hornotini teretes, veteriores longitudinaliter striati vel sulcati, cortice albescente nitido, cicatricibus orbicularibus grandis obsiti. Internodii 5—10 mm longi. Stipulae interpetiolares annulares, connatae, 4—5 mm longae, truncatae, apice

breviter apiculatae, adpresse denseque strigosae, resinosae. Folia subsessilia, petiolis 1--3 mm longis, resinosis puberulisque suffulta, oblongo-lanceolata inferne longe attenuata, basi ipso obtusa vel breviter rotundata, antice longe acuminata et cuspidata, apice ipso acuta, 8--16 cm longa et 2,5--4 cm lata, utrinque opaca, subtus pallidiora, barbata et ad nervos adpresse sericea, in axillis nervorum piloso-puberula, nervo medio supra in sulco prominulo, subtus carinato-prominente, lateralibus utroque latere 7--11, rectangulariter abeuntibus et apicem versus arcuatis, supra impressis, subtus prominentibus reticulatione conspicua, margine revoluta, subcoriacea vel coriacea.

Inflorescentiae axillares, cymoso-bifidae. Pedunculus glaber, 4--6,5 cm longus, rami glabri vel strigosi et resinoso-punctati 3--4 cm longi, 10--14-flori. Flores numerosi, sessiles, tubus calycis obovatus, strigillosus, 1 mm longus, apice truncatus, lobi calycis nulli. Corolla 3--4 mm longa, extus albo-strigosa, tubus 3 mm longus, lobi 4 mm longi. Stamina 4, sub medio tubae affixa, filamenta 0,7 mm longa, glabra, antherae lineares, 2,5 mm longae, e fauce brevissime exsertae. Stylus 3,5 mm longus, stigmata 4-dentata. Fructus sessilis, ellipticus, 4--5 mm longus, valde resinoso-punctatus, 4-locularis, drupaceus, 4-spermus. Ovarium 4-loculare.

Holotypus: BISSE et al. 26856 HAJB. Cuba, Prov. Guántanamo, Sierra de Moa, orillas de Rio Báez, prope al campo Los Naranjos. 01.08.1975. — Isotypi: HAC, JE, BP.

Obs.: Stenostomo resinoso (Vahl) Griseb. affinis, quae a specie nostra foliis longiore (2--8 mm) longe petiolatis, minoribus, apice obtusis, supra lucidis, margine planis, ramulis cymae multto brevioribus (1 cm) 5--8-floris, sine dubio specifice differt.

Stenostomum imbricatum Borhidi sp. nova

(Syn.: Antirhea imbricata Borhidi in schaedis)

Frutex parvus. Rami veteriores cinereo-nigrescentes, in sicco crassiuscula striato-plicati, hornotini in sicco nigrescentes, 4-anguli vel leviter 4-alati, glabri, ad apicem densissime foliigeri, resinam abunde exsudantes, internodiis 0,5--1,0 mm longis. Stipulae interpetiolares ovatae vel triangulares, crassiuscule coriaceae, apice acutae, dorso longitudinaliter sulcatae, 2 mm longae, deciduae. Folia 0,5--1,0 mm longe petiolata, petiolis circumcirca anguste alatis, ventrifixis, ad ramulos superficie adaxiali petiolis adnatis suffulta, obovata, inferne sensim angustata, basi acuta, apice rotundata, 1--2,2 cm longa et 0,5--1 cm lata,

nervo medio supra tenuiter et profunde impresso, supra medium evanescente subtus bene prominente, lateralibus utroque latere 2—3, utrinque inconspicuis, lamina supra ad marginem versus brevissime pilosa demum glabra, margine recurva, coriacea.

Inflorescentiae in axillis foliorum superiorum sub apice ramorum sessiles, uniflorae. Pedunculus nullus. Calyx totus 4,5—5 mm longus; tubus cylindraceus, limbo libero 3 mm longo, ovario 1,5—2-plo longiore, superne non ampliato. Lobi plerumque 4, semiorbiculares vel late triangulares, 0,5—1 mm longi, glabri, tubo 4—5-plo breviores. Corolla alba, tubus cylindraceus, 4—6 mm longus, 2 mm crassus, intus glaber. Stamina 5, fauce inserta, subsessilia, antherae lineares, 3 mm longae, basi breviter sagittatae. Ovarium 2 mm longum, 3—4-loculare, locula 1—2 aborta plerumque loculis fertilibus 2 suffultum, ovula solitaria in quoque loculo. Fructus oblongus, extrorse curvatus, angulatus tubo calycino persistente apice coronatus, 8—10 mm longus, 3—4 mm crassus, bilocularis, bispermus.

Holotypus: BISSE 21349 HAJB. Cuba. Oriente, Prov. Santiago, Sierra Maestra, El Uvero, pluviisilva del Alto de la Francia. 05.02.1972.

Obs.: Stenostomo abbreviato (Urb.) Borhidi & Fernández affinis, sed ab ea petiolis ventrifixis, foliis basi acutis vel obtusis, nervis lateralibus utroque latere 2—3, inflorescentiis sessilibusque 1-floris atque floribus duplo minoribus, ovario 3—4-loculare clare differt.

Stenostomum reticulare Borhidi & M.Z. Fernandez **sp. nova**

(Syn.: Antirhea reticularis Borhidi & Fernandez in schaedis)

Frutex vel arbor parva. Rami hornotini 4-anguli, glabri, cortice brunneo, veteriores longitrorse striati et fissurati, grisei. Stipulae interpetiolares late triangulares, apice breviter mucronatae, 2 mm longae et latae dorso adpresse ferrugineo-sericeae. Folia ad apicem ramorum fasciculata, petiolis 3—7 mm longis glabribusque suffulta, lamina elliptica, 2,5—5 cm longa et 1—2 cm lata, apice rotundata, basi obtusa vel rotundata, leviter obliqua, in petiolum abrupte decurrens, margine integra, membranacea vel subchartacea; nervo medio supra leviter impresso, supra medium applanato vel levissime prominente, subtus prominulo, lateralibus utroque latere 3-4, supra tenuiter impressis, subtus prominulis, ante marginem evanescentibus, utrinque dense et prominenter reticulatis, supra punctis prominulis dense interjectis granulata, subtus nervis tertiariis densissime reticulata, in axillis nervorum lateralium pilis longis sparsisque domatiata, ceterum glaberrima.

Inflorescentiae axillares 2—5, ab axillis foliorum superiorum fasciculatim abeuntes, 1,5—2,5 cm longe pedunculatae, apice bifidae. Rami laterales cymae 0,5—1,5 cm longae, 4—6-florae. Pedunculus lateraliter compressus, alatus, glaberrimus. Flores sessiles biseriatim dispositi; calycis tubus extus adpresse pilosus, 1,0—1,5 mm longus, apice truncatus vel brevissime et obscure 4-lobulatus; discus e tubo paullo emergens, glaber; corolla 4—5 mm longa, tubus 4-angulus, 3—4 mm longus, basi adpresse sericeus; lobi 4, ovati, 0,5—1 mm longi, glabri, margine anguste membranacei et sparse pilosi. Stamina 4, in tubo corollinö supra medio affixa, filamenta subnulla, glabra, antherae ovatae. Ovarium biloculare. Fructus non visus.

Holotypus: CLEMENTE 4513. HAC. Cuba Orientalis, Moa, camino de la Mina Delta, puente del Rio Cayoguán. Leg.: 04.07.0945. Isotypus: HAC.

Obs.: Antirheae urbanianae C.T. White affinis, a qua species nostra foliis ellipticis, membranaceis vel subchartaceis, utrinque dense reticulatis, supra granulatis, subtus domatiatis, atque ramis cymae paucifloris certe specifice differt.

Stenostomum revolutum Borhidi **sp. nova**

(Syn.: Antirhea monantha Borhidi in schaedis)

Frutex parvus, valde ramosus. Rami veteriores albicantes vel pallide cinerei, cylindracei, plicato-striati, hornotini 4-anguli, in sicco brunneo-nigrescentes, pilis minutis adpressis strigulosi et punctis resinosis emergentibus scabriusculi, ad apicem dense foliigeri, resinam exsudantes, internodiis plerumque 0,5—2 mm longis, rariter longioribus. Stipulae interpetiolares crassiuscule coriaceae, oblongo-ovatae, 2—3,5 mm longae, concavae, acutae, apice acutae acuminataeque saepe horizontaliter patentes, adpresse puberulae demum deciduae. Folia subsessilia, usque ad 1,5 mm longe petiolata, petiolis crassis strigilloso-puberulis suffulta, lamina late obovata, inferne sensim angustata, basi ipsa acuta vel obtusa, apice rotundata, 0,6—1,4 cm longa, 0,4—1,0 cm lata, nervo medio supra leviter impresso, supra medium evanescente, subtus prominente, lateralibus supra nullis, subtus utroque latere 5—8, tenuiter impressis, reticulato-conjunctis, plerumque inconspicuis, margine in statu juvenili sparse pilosa, caeterum glabra, margine valde revoluta, crasse coriacea.

Inflorescentiae in axillis foliorum superiorum abeuntes 1-florae. Pedunculus 1—3 mm longus, crassus, glaber, posterior lignescens, ad ramos veteriores permanens. Calyx 3—4 mm longus, tubus obovatus, glaber, superne bilobatus, lobi 0,5—1 mm longi, late triangulares, acuti vel obtusi. Co-

rolla alba, tubus leviter angulatus, 10—12 mm longus, 11 mm crassus, intus glaber, lobi 5, obovati, vel ovati, basi auriculato-cordati, tubo 3--4-plo breviores. Stamina 5, fauci affixa, inclusa, filamenta subnulla, antherae oblongo-lineares, 2--2,5 mm longae. Stylus 3--4 mm longus, aequilatus, glaber, apice 1--2 mm longe ramificatus, bilobus. Discus annularis 0.5 mm altus, glaber. Ovarium 4-loculare, loculis 3 abortis, ovulum solitarium. Fructus non visus.

Holotypus: BISSE & al. 47824, HAJB. Cuba, Oriente, Prov. Guantanamo, La Tinta, Peladeros de Jauco, cerca de Guajimero. 06.06.1982.

Obs.: Ab Stenostomo orbiculari (Alain) Borhidi & Fernandez stipulis oblongo ovatis, apice acutis, 2—3,5 mm longis, foliis obovatis, basi attenuatis, margine valde revolutis, nervis lateralibus utrinque nullis differt.

Clave analítica para las especies cubanas

1 a Plantas con hojas de hasta 4 cm de largo 2
b Plantas con hojas mayores 13
2 a Hojas aristado mucronadas en el ápice 3
b Hojas no aristado-mucronadas 4
3 a Plantas resinosas. Hojas de nerviación muy aparente y sobresaliente en el haz, pedunculos 1--3-floros, lóbulos del cáliz oblongo-obovados de 1,5—2 mm. Fruto de 1,5 cm de largo .... **1. S. aristatum**
b Planta no resinosa. Hojas muy revolutas con una nerviación muy densa pero no aparente y sobresaliente en el haz; pedunculos 1-floros, lóbulos del cáliz deltoideos, muy cortos. Fruto de 8--9 mm .... **2. S. mucronatum**
4 a Plantas mayormente resinosas. Inflorescencias 1--2-floras, pedunculo simple o muy brevemente bifurcado, corto 5
b Inflorescencias 3-multifloras; pedunculo ramificado con ramas 2, a plurifloras 12
5 a Inflorescencias bifurcadas, 2-floras 6
b Inflorescencias 1-floras 7
6 a Hojas aovadas a obovadas u obcordatas de 0.8--2.5 cm de ancho, nervios laterales conspicuos en ambas caras, flores de 10—12 mm de largo .... **3. S. abbreviatum**
b Hojas oblongo-elípticas, de 0.4—0.7 cm de ancho, nervios laterales inconspicuos en el haz, flores 4—6 mm de largo .... **4. S. biflorum**

7 a Flores brevemente pedunculadas, pedunculos persistentes 8
b Flores sésiles 10
8 a Plantas resinosas, no espinosas, pedunculo muy corto 9
b Planta no resinosa con ramas espinosas, pedunculo de hasta 1 cm, hojas reticulado venosas en el haz .... **5. S. minutifolium**
9 a Planta resinosa, estípulas anchamente triangulares o semiorbiculares de 1—2 mm de largo, hojas orbiculares, redondeadas a subacorazonadas en la base, el margen plano, nervios laterales aparentes .... **6. S. orbiculare**
b Estípulas oblongo-aovadas de 2—3.5 mm, hojas obovadas, estrechadas en la base, el margen muy revoluto, nervios laterales no conspicuos .... **7. S. revolutum**
10 a Ramitas pelosas, hojas elípticas, nervios laterales conspicuos en ambas caras 11
b Ramitas glabras, hojas obovadas sin nervios aparentes en ambas caras .. .. **8. S. imbricatum**
11 a Hojas sentadas, orbiculares de 15—30 mm de largo, acorazonadas en la base .... **9. S. nipense**
b Hojas elípticas u obovadas de 2—8 cm de largo estrechadas en el pecíolo, estípulas semiorbiculares, fruto de 10—12 mm . **10. S. baracoense**
12 a Planta resinosa, hojas de 1—2.5 cm, agudas con 4—6 nervios laterales, flores 4-meras .... **11. S. myrtifolium**
b Planta no resinosa, hojas de 2—4 cm, obovadas, redondeadas en el ápice, nervios laterales muy numerosos, flores 5-meras **12. S. ophiticola**
13 a Plantas resinosas 14
b Plantas no resinosas 17
14 a Flores solitarias, sentadas en las axilas de las hojas elípticas u obovadas de 2—9 cm. Estípulas semiorbiculares, leñosas, connadas, ciliadas en el margen .... **10. S. baracoense**
b Flores en cimas bifurcadas 15
15 a Inflorescencias pelosas, hojas aovadas a elípticas, espaciado-pelosas, no domaciadas en el envés .... **13. S. apiculatum**
b Hojas oblongo-lanceoladas, domaciadas, inflorescencias glabras 16
16 a Hojas cartáceas, pecioladas, pedunculo delgado, ramas de 1 cm, 5—8-floros .... **14. S. densiflorum**
b Hojas coriáceas sésiles, pedunculo grueso, ramas más largas de 10—14-floros .... **15. S. cuspidatum**
17 a Hojas granulosas cuando secas 18

b Hojas no granulosas 19

18 a Hojas con nervios poco aparentes, granulosas en el envés ....
.... **16. S. granulatum**

b Hojas con una nerviación muy reticulada, granulosas en el haz ....
.... **17. S. reticulatum**

19 a Lóbulos del cáliz 5, conspicuos, ciliados o estrigilosos 20

b Lóbulos del cáliz 4, o truncados 21

20 a Ramitas pelosas, hojas reticuladas, ovario 6—9-locular ....
.... **18. S. rotundatum**

b Ramitas glabras, hojas brillantes, ovario 2-locular .... **19. S. lucidum**

21 a Cáliz y corola densamente albo-seriçeos, nervio medio barbado en el envés .... **20. S. radiatum**

b Cáliz y corola glabros o esparcido-pelosos, nervio medio no barbado en el envés 22

22 a Hojas obovadas con nervios laterales muy numerosos 23

b Hojas con nervios laterales espaciados 24

23 a Flores sentadas en las ramas de la inflorescencia ... **21. S. multinerve**

b Flores pediceladas .... **22. S. pedicellare**

24 a Hojas con 3--4 pares de nervios laterales .... **23. S. maestrense**

b Nervios laterales 6—10 pares 25

25 a Nervios laterales hundidos en el haz 26

b Nervios laterales aplanados o algo prominulos en el haz 27

26 a Hojas de 4--7 cm con hoyitos (escrobiculos) en las axilas de los nervios en el envés .... **24. S. scrobiculatum**

b Hojas de 4--15 cm, sin hoyitos en el envés .... **25. S. shaferi**

27 a Corola pelosita por fuera, estípulas sericeas ...... **26. S. urbanianum**

b Corola y estípulas glabras .... **27. S. occidentale**

Conspectus de las especies cubanas

1. **Stenostomum aristatum** Britt. Bull. Torr. Bot. Cl. 44: 36. 1917.
   Holotype: Britt. & Cowell 13241, NY!, isotypes: GH!, HAC!, US!
   Syn.: Antirhea aristata Urb. Ark.f.Bot. 21A. No. 5: 83. 1927.
   Area: Cuba: VC, CA, Cam, Tu, Ho, SC, endémica
2. **Stenostomum mucronatum** (Urb.) Borhidi & M.Z. Fernandez comb. nova
   Basionym: Antirhea mucronata Urb. Symb. Antill. 1: 440. 1899.
   Holotype: Wr. 2782 GOET.!, isotypes: GH, HAC!, MBG!, NY!, S!
   Area: Cuba: Ho (Sierra de Nipe), endémica

3. **Stenostomum abbreviatum** (Urb.) Borhidi & M.Z. Fernandez comb. nova

Basionym: Antirhea abbreviata Urb. Symb. Antill. 9: 159. 1923.

Lectotype: EKMAN 9523, S!

Area: Cuba: Ho, SC, Gu. endémica

3/a. — ssp. **abbreviatum**

3/b. — ssp. **moaense** (M.Z. Fernandez) Borhidi & M.Z. Fernandez comb. et stat. novus

Basionym: Antirhea abbreviata var. moaensis M.Z. Fernandez Acta Bot. Acad. Sci. Hung. 28: 87. 1982.

Holotype: CLEMENTE 3639. HAC!

3/c. — ssp. **obcordata** (Alain) Borhidi & M.Z. Fernandez comb. nova

Basionym: Antirhea obcordata Alain Candollea 17: 108. 1960.

4. **Stenostomum biflorum** Borhidi Acta Bot. Hung. 38: 145. 1995.

Holotype: BISSE & KÖHLER 7091 HAJB!, isotypes: HAC!

Syn.: Antirhea biflora Borhidi in schaedis

Area: Cuba SC (Sierra de Cristal), endémica

5. **Stenostomum minutifolium** (Borh. & Cap.) Borhidi & M.Z.Fernandez comb. nova

Basionym: Antirhea minutifolia Borhidi & Capote Abstracta Bot. Budapest 7: 40. 1977.

Holotype: BORHIDI 27803 HAC!, isotype: BP!

Area: Cuba, Tu, Ho, SC, endémica

6. **Stenostomum orbiculare** (Alain) Borhidi & M.Z. Fernandez comb. nova

Basionym: Antirhea orbicularis Alain Contr. Ocas. Mus. Hist. Nat de la Salle, 17: 2. 1959.

Holotype: CLEMENTE 6219 LS/HAC!, isotype: NY!, US!

Area: Cuba, H. (Moa), endémica

7. **Stenostomum revolutum** Borhidi Acta Bot. Hung. 38: 148. 1994.

(Syn.: Antirhea monantha Borhidi in schaedis)

Area: Cuba, Gu. (Peladeros de Jauco), endémica

8. **Stenostomum imbricatum** Borhidi Acta Bot. Hung. 38: 146. 1995.

Holotype: BISSE 21349 HAJB!

Syn.: Antirhea imbricata Borhidi in schaedis

Area: Cuba, SC (Sierra Maestra), endémica

9. **Stenostomum nipense** (Borhidi & Muñiz) Borhidi & M.Z. Fernandez comb. nova

Basionym: Antirhea nipensis Borhidi & Muñiz Abstracta Bot. Budapest 7: 41. 1977.

Holotype: SV 27798 HAC!

Area: Cuba: Ho. (Sierra de Nipe), endémica

10. **Stenostomum baracoense** Borhidi Acta Bot. Hung. 38: 144. 1995.

Holotype: BISSE & al. 36878 HAJB!

Syn.: Antirhea baracoensis Borhidi in schaedis

Area: Cu: Gu. (Baracoa)

11. **Stenostomum myrtifolium** Griseb. Fl.Br.W.I. 1861: 334.

Holotype: BRACE 445, GOET.

Syn.: Antirhea myrtifolia Urb. Symb. Antill. 1: 440, 1899.

Area: Cuba: CA, Cam.; Bahamas

12. **Stenostomum ophiticola** (Alain) Borhidi & M.Z. Fernandez comb. nova

Basionym: Antirhea ophiticola Alain Contr. Ocas. Mus. Hist. Nat. de la Salle, 17: 1, 1959.

Holotype: ACUÑA & ZAYAS 19806 SV/HAC!, isotype: LS/HAC.

Area: Cuba: Ho, SC, endémica

13. **Stenostomum apiculatum** Britt. & Standl. Bull. Torr. Bot. Cl. 50: 50. 1923.

Holotype: LEÓN 10806, NY!, isotypes: HAC!, US!

Syn.: Laugeria apiculata Urb. & Ekm. Ark.f.Bot. 20A No. 5: 58. 1926.

Terebraria apiculata Alain Contr. Ocas. Mus. Hist. Nat de la Salle 17: 11. 1959.

Neolaugeria apiculata Nicolson Brittonia 31: 120. 1979.

Area: Cuba: Gr, SC (Sierra Maestra), endémica

14. **Stenostomum densiflorum** Wr. ex Griseb. Cat. Pl. Cub. 1866: 132.

Type: Wr. 2713. Cuba occ. GH, isotype: HAC!, NY

Syn.: Guettarda densiflora Maza Anal. Soc. Esp. Hist. Nat. 23: 290. 1895.

Laugeria densiflora Hitchc. Rep. Mo. Gard. 4: 93. 1893.

Neolaugeria densiflora Nicolson Brittonia 31: 121. 1979.

Area: Cuba: Gr, SC, Ho, Gu. Española, Puerto Rico, Bahamas

15. **Stenostomum cuspidatum** Borhidi Acta Bot. Hung. 38: 145. 1995.

Syn.: Terebraria cuspidata Borhidi in schaedis

Area: Cuba: Gu. (Baracoa), endémica

16. **Stenostomum granulatum** Griseb. Mem. Amer. Acad. Nov. Ser. 8: 507. 1862.

Holotype: Wr. 1272 GOET.!, isotypes: GH, GAC!, NY!

Syn.: Antirhea granulata Urb. Symb. Antill. 1: 439. 1899.

Area: Cuba: Gu (Monteverde), Española

17. Stenostomum reticulare Borhidi & M.Z. Fernandez Acta Bot. Hung. 38: 147. 1994.

(Syn.: Antirhea reticularis Borhidi & Fernandez in schaedis)

Area: Cuba: Ho (Moa), endémica

18. Stenostomum rotundatum Griseb. Cat. Pl. Cub. 1866: 132.

Holotype: Wr. 2714 GOET!, isotype: GH, HAC!, MBG!, NY!, S!, US!

Syn.: Antirhea rotundata Hook.f. in Benth. & Hook. Gen.Pl. 2: 100. 1873.

Area: Cuba: PR, IJ, endémica

19. Stenostomum lucidum (Sw.) C.F. Gaertn. Fruct. 3: 69, 1805.

Basionym: Laugieria lucida Sw. Prodr. 1788: 48.

Holotype: Sw. s.n. S!

Syn.: Antirhea lucida Hook.f. in Benth. & Hook. Gen.Pl. 2: 100. 1873.

Area: Cuba e IJ, Bahamas, Antillas, Honduras

20. Stenostomum radiatum Griseb. Cat.Pl. Cub. 1866: 132.

Holotype: Wr. 2712. GOET. Isotype: GH, MBG!, NY!, S!, US!

Syn.: Antirhea radiata Urb. Symb. Antill. 1: 435. 1899.

Stenostomum pauciflorum Wr. in Sauv. Anal. Acad. Habana 6: 67. 1869.

Area: Cuba: PR, VC, Ci, SS, Gr, SC, Ho, Gu; Española

21. Stenostomum multinerve (Urb.) Borhidi & M.Z. Fernádez comb. nova

Basionym: Antirhea multinervis Urb. Symb. Antill. 9: 159. 1923.

Lectotype: EKMAN 3598 S!, holotype: B^+.

Area: Cuba: Ho (Moa), endémica

22. Stenostomum pedicellare (Borhidi & Bisse) Borhidi & M.Z. Fernandez comb. nova

Basionym: Antirhea pedicellaris Borhidi & Bisse Acta Bot. Acad. Sci. Hung. 28: 87. 1982.

Holotype: HAJB 17793 Bisse & Lippold, isotype: JE.

Area: Cuba: Ho (Moa), endémica.

23. Stenostomum maëstrense (Urb.) Borhidi & M.Z. Fernandez comb. nova

Basionym: Antirhea maëstrensis Urb. Symb. Antill. 9: 528. 1928.

Holotype: EKMAN 15849 B^+. Lectotype: S!, isolectotypes: NY!, US!

Area: Cuba: Gr, SC (Sierra Maestra), endémica

24. Stenostomum scrobiculatum (Urb.) Borhidi & M.Z. Fernandez comb. nova

Basionym: Antirhea scrobiculata Urb. Symb. Antill. 9: 529. 1928.

Holotype: EKMAN 15880 B^+, lectotype: S!, isolectotype: NY!

Area: Cuba: Ho, SC, Gu, endémica

25. Stenostomum shaferi (Urb.) Borhidi & M.Z. Fernandez comb. nova

Basionym: Antirhea shaferi Urb. Feddes. Repert. 13: 479. 1915.

Holotype: SHAFER 8142 B+, lectotype: SHAFER 3160 NY!, isolectotypes: HAC!, US!

Area: Cuba: Ho, SC, Gu, endémica

26. Stenostomum urbanianum (C.T. White) Borhidi & M.Z. Fernandez comb. nova

Basionym: Antirhea urbaniana C.T. White J. Am. Arb. 27: 121. 1946.

Holotype: Wr 2710 GOET.!, lectotype: GH, HAC!, MBG!, NY!, S!

Syn.: Antirhea tenuiflora Urb. Symb. Antill. 1: 438. 1899. non F. Muell.

Area: Cuba: PR, IJ, Hab, Mat, VC, Ci, SS, endémica

27. Stenostomum occidentale (Urb.) Borhidi & M.Z. Fernandez comb. nova

Basionym: Antirhea occidentalis Urb. Symb. Antill. 9: 529. 1928.

Holotype: EKMAN 11913 B+, lectotype: S!

Area: Cuba: PR, IJ, Hab. endémica

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THE GENUS STENOSTOMUM C.F. GAERTN. (RUBIACEAE) OR THE RECONSIDERATION OF THE NEW WORLD ANTIRHEA SPECIES

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(Received: 15 January, 1994)

A new taxonomic treatment of the Old World species of the genus Antirhea Juss. by CHAW and S. DARWIN suggested the separation of this group from the New World representatives at generic level. This important result makes necessary the re-consideration of the New World species and the restoration of the genus Stenostomum F.G. Gaertn. Within the New World genus two sections are recognized: sect. Stenostomum and sect. Resinanthus with resinous leaves, buds and flowers and a dense secondary venation of the leaves. The Antillean genus Neolaugeria Nicols. is merged again into Stenostomum and treated as its third section. Pittoniotis Griseb. and Ottoschmidtia Urb. two monotypic genera of the Caribbean closely related to Stenostomum are to be maintained as separate units.

Introduction

The excellent systematic studies published by CHAW and S. DARWIN (1992) on the Old World species of the genus Antirhea Juss. ex. Commers. and by S. Darwin on the genus Timonius, put in a completely new light the taxonomic position and the generic delimitation of the New World Antirheas. The mentioned authors include into Antirhea Juss. s.str. 36 Old World species distributed from Madagascar to China, and through SE. Asia, the Philippines, Malaysia, Sunda Islands and New Guinea to Australia and the South Pacific Islands. They are not resinous trees and shrubs characterized by unisexual dioecious bracteate flowers with manifested sexual dimorphism and arranged in dichasial cymes. Female inflorescence is frequently reduced to solitary flowers. Calyx with well-performed persistent lobes. Corolla 3- or 4-lobed, tube is commonly hairy or bearded inside at the base or in the lower part, sometimes naked. Pollen grains are semitectate, inaperturate to 1-porate.

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The resurrection of the genus Stenostomum

The new detailed description of the genus Antirhea made necessary to revise the character combination of the New World Antirheas including about other 36 species in the Caribbean, with a strong diversification center in Cuba and Hispaniola. They are mostly resinous trees and shrubs with bisexual -- mostly ebracteate -- flowers without sexual dimorphism. Calyx lobes mostly not developed or truncate. Corolla 4- to 5-lobed, tube is commonly naked inside or hairy in the throat or in the upper part. Pollen grains are tectate, punctitegillate, 3-colporate.

These important differences make necessary the re-consideration of the New World species and the restoration of the genus Stenostomum F.G. Gaertn. Within the New World genus two sections are recognized: sect. Stenostomum and sect. Resinanthus with resinous leaves, buds and flowers and a dense secondary venation of the leaves. The Antillean genus Neolaugeria Nicols. is merged again into Stenostomum and treated as its third section. Pittoniotis Griseb. and Ottoschmidtia Urb. two monotypic genera of the Caribbean closely related to Stenostomum are to be maintained as separate units.

The taxonomic subdivision of the genus is suggested as follows:

I. Sectio: Stenostomum. Arbores vel frutices non resinosi, foliis majoribus foliaceis vel chartaceis, rariter coriaceis stipulis liberis, stigmatibus 2-3-lobulatis. Typus sectionis: Laugieria lucida Sw.

Species huc pertinentes: S. cahosianum, S. coriaceum, S. granulatum, S. jamaicense, S. lucidum, S. maëstrense, S. minutifolium, S. mucronatum, S. multinerve, S. obtusifolium, S. occidentale, S. oliganthum, S. ophiticola, S. pedicellare, S. pitonianum, S. portoricense, S. radiatum, S. reticulate, S. rotundatum, S. scrobiculatum, S. shaferi, S. sintenisii, S. tomentosum, S. urbanianum.

II. Sectio: Resinanthus Borhidi sect. nova: Frutices vel arbores parvi resinosi, inflorescentiis abbreviatis dense bracteosis, foliis plerumque minoribus, coriaceis, stipulus liberis vel breviter connatis, stigmatibus 2-3-lobulatis. Typus: sectionis: Artirhea abbreviata Urb.

Species huc pertinentes: S. abbreviatum, S. acutatum, S. albobruneum, S. aristatum, S. baracoense, S. biflorum, S. ekmanii, S. ellipticum, S. heteroneurum, S. imbricatum, S. montecristinum, S. myrtifolium, S. nipense, S. orbiculare, S. revolutum.

III. Sectio: Neolaugeria (Nicols.) Borhidi sect. & comb. nova: Frutices vel arbores valde resinosi, stipulis connatis in anillum truncatum, stigmatibus 4-5-lobulatis. Typus sectionis: Laugeria resinosa Vahl.

Species hus pertinentes: S. apiculatum, S. cuspidatum, S. densiflorum, S. hotteanum, S. lineolatum, S. resinosum.

Conspectus specierum

Stenostomum abbreviatum (Urb.) Borhidi & M.Z. Fernandez Acta Bot. Hung. 38: 152. 1995.

Basionym: Antirhea abbreviata Urb. Symb. Antill. 9: 159. 1923. Lectotype: EKMAN 9523, S!

-- ssp. **abbreviatum**

-- ssp. **moaense** (M.Z. Fernandez) Borhidi & M.Z. Fernandez l.c. 152. 1995.

Basionym: Antirhea abbreviata var. moaensis M.Z. Fernandez Acta Bot. Acad. Sci. Hung. 28: 87. 1982.

Holotype: CLEMENTE 3639. HAC!

-- ssp. **obcordata** (Alain) Borhidi & M.Z. Fernandez l.c. 152. 1995.

Basionym: antirhea obcordata Alain Candollea 17: 108. 1960.

Stenostomum acutatum DC. Prodr. 4: 460. 1930.

Syn.: Stenostomum viscosum Griseb. Fl.Br.W.I. 1861: 334.

Antirhea acutata Urb. Symb. Antill. 1: 439. 1899.

-- var. **latifolium** (Urb.) Borhidi comb. nova

Basionym: Antirhea acutata var. latifolia Urb. Symb. Antill. 1: 439. 1899.

Type: SINTENIS 3180 (B^+), lectotype: US!

Stenostomum albobruneum (Urb. & Ekm.) Borhidi comb. nova

Basionym: Antirhea albobrunea Urb. & Ekm. Ark.f.Bot. 21 A. No. 5: 83. 9127.

Holotype: EKMAN H 4146 S!, isotypes: GH!, US!

Stenostomum apiculatum Britt. & Standl. Bull. Torr. Bot. Cl. 50:50. 1923.

Holotype: LEÓN 10806, NY!, isotypes: HAC!, US!

Syn.: Laugeria apiculata Urb. & Ekm. Ark.f. Bot. 20A No. 5: 58. 1926.

Terebraria apiculata Alain Contr. Ocas. Mus. Hist. Nat de la Salle 17: 11. 1959.

Neolaugeria apiculata Nicolson Brittonia 31: 120. 1979.

Stenostomum aristatum Britt. Bull. Torr. Bot. Cl. 44: 36. 1917.

Holotype: Britt. & Cowell 13241, NY!, isotypes: GH!, HAC!, US!

Syn.: Antirhea aristata Urb. Ark.f.Bot. 21A. No. 5: 83. 1927.

Stenostomum aromaticum (Castillo-Campos & Lorence) Borhidi comb. nova

Basionym: Antirhea aromatica Castillo-Campos & Lorence Ann. Mo. Bot. Gard. 72: 268. 1985.

Holotype: CASTILLO-CAMPOS 1957, XAL, isotypes: MEXU, XAL.

Stenostomum baracoense Borhidi Acta Bot. Hung. 38: 144. 1995.

Holotype: BISSE & al. 36878 HAJB.

Syn.: Antirhea baracoensis Borhidi in schaedis

Stenostomum biflorum Borhidi Acta Bot. Hung. 38: 145. 1995.

Holotype: BISSE & KÖHLER 7091 HAJB!, isotypes: HAC!

Syn.: Antirhea biflora Borhidi in schaedis

Stenostomum cahosianum (Urb. & Ekm.) Borhidi comb. nova

Basionym: Antirhea cahosiana Urb. & Ekm. Ark.f.Bot. 22A No. 10: 93. 1929.

Holotype: EKMAN, H 8537/a S!, isotypes: A!, NY!

Stenostomum coriaceum (Vahl) Griseb. Fl.Br.W.I. 1861: 334.

Basionym: Laugeria coriacea Vahl. Eclog. 1: 26. 1796.

Syn.: Stenostomum dichotomum DC. Prodr. 4: 461. 1830.

Antirhea coriacea Urb. Symb. Antill. 1: 436. 1899.

Stenostomum cuspidatum Borhidi Acta Bot. Hung. 38: 145. 1995.

Syn.: Terebraria cuspidata Borhidi in schaedis

Stenostomum densiflorum Wr. ex Griseb. Cat.Pl.Cub. 1866: 132.

Type: Wr. 2713. Cuba occ. GH, isotype: HAC!, NY

Syn.: Guettarda densiflora Maza Anal. Soc. Esp. Hist. Nat. 23: 290. 1895.

Laugeria densiflora Hitchc. Rep. Mo. Gard. 4: 93. 1893.

Neolaugeri densiflora Nicolson Brittonia 31: 121. 1979.

Stenostomum ekmanii (Borhidi) Borhidi comb. nova

Basionym: Antirhea ekmanii Borhidi Acta Bot. Hung. 29: 193. 1983.

Holotype: EKMAN, H 15429 S!, isotype: NY!

Stenostomum ellipticum (Urb. & Ekm.) Borhidi comb. nova

Basionym: Antirhea elliptica Urb. & Ekm. Ark.f.Bot. 24A No. 4: 52. 1932.

Holotype: EKMAN, H 9863 S!, isotypes: GH!, NY!, S!, US!

Stenostomum granulatum Griseb. Mem. Amer. Acad. Nov. Ser. 8: 507. 1862.

Holotype: Wr. 1272 GOET.!, isotypes: GH, HAC!, NY!

Syn.: Antirhea granulata Urb. Symb. Antill. 1: 439. 1899.

Stenostomum heteroneurum (Urb. & Ekm.) Borhidi comb. nova

Basionym: Antirhea heteronerua Urb. & Ekm. Ark.f.Bot. 21A No. 5: 83. 1927.

Holotype: EKMAN, H 3843 S!

Stenostomum hotteanum (Urb.) Borhidi comb. nova

Basionym: Laugeria hotteana Urb. Ark.f.Bot. 20A No. 5: 58. 1926.

Holotype: EKMAN, H 601 S!

Syn.: Terebraria hotteana Alain Brittonia 20: 161. 1968.

Neolaugeria apiculata Nicols. p.p. Brittonia 31: 120. 1979.

Neolaugeria hotteana Borhidi Acta Bot. Hung. 37: 85. 1993.

Stenostomum imbricatum Borhidi Acta Bot. Hung. 38: 146. 1995.

Holotype: BISSE 21349 HAJB!

Syn.: Antirhea imbricata Borhidi in schaedis

Stenostomum involucratum (Urb. & Ekm.) Borhidi comb. nova

Basionym: Antirhea involucrata Urb. & Ekm. Ark.f.Bot. 21A No. 5: 84. 1927.

Holotype: EKMAN, H 3527 S!, isotypes: A!, US!

Stenostomum jamaicense (Urb.) Borhidi comb. nova

Basionym: Antirhea jamaicensis Urb. Symb. Antill. 1: 435. 1899.

Holotype: HARRIS 5805 (B+)

Syn.: Stenostomum bifurcatum Griseb. Fl.Br.W.I. 1861: 333. non DC.

Antirhea bifurcata Hook.f. in Benth. & Hook. Gen.Pl. 2: 100. 1873.

Stenostomum lineolatum (Urb.) Borhidi comb. nova

Basionym: Laugeria lineolata Urb. Ark.f.Bot. 20A No. 5: 58. 1926.

Holotype: EKMAN, H 1224 S!, isotype: US!

Syn.: Terebraria lineolata Alain Brittonia 20: 161. 1968.

Neolaugeria apiculata Nicols. Brittonia 31: 120. 1979.

Neolaugeria lineolata Borhidi Acta Bot. Hung. 37: 85. 1993.

Stenostomum lucidum (Sw.)C.F. Gaertn. Fruct. 3: 69. 1805.

Basionym: Laugieria lucida Sw. Prodr. 1788: 48.

Holotype: Sw. sn.n. S!

Syn.: Antirhea lucida Hook.f. in Benth. & Hook. Gen.Pl. 2: 100. 1873.

Stenostomum maëstrense (Urb.) Borhidi & M.Z. Fernandez l.c. 154. 1995.

Basionym: Antirhea maestrensis Urb. Symb. Antill. 9: 528. 1928.

Holotype: EKMAN 15849 B+. Lectotype: S!, isolectotypes: NY!, US!

Stenostomum minutifolium (Borh. & Cap.) Borhidi & M.Z. Fernandez l.c. 152. 1995.

Basionym: Antirhea minutifolia Borhidi & Capote Abstracta Bot. Budapest 7: 40. 1977.

Holotype: BORHIDI 27803 HAC!, isotype: BP!

Stenostomum montecristinum (Urb.) Borhidi comb. nova

Basionym: Antirhea montecristina Urb. Ark.f.Bot. 24A No. 4: 51. 1932.

Holotype: EKMAN, H 13142 S!, isotypes: GH!, NY!

Stenostomum mucronatum (Urb.) Borhidi & M.Z. Fernandez l.c. 151. 1995.

Basionym: Antirhea mucronata Urb. Symb. Antill. 1: 440. 1899.

Holotype: Wr. 2782 GOET.!, istoype: GH, HAC!, MBG!, NY!, S!

Stenostomum multinerve (Urb.) Borhidi & M.Z. Fernandez l.c. 154. 1995.

Basionym: Antirhea multinervis Urb. Symb. Antill. 9: 159. 1923.

Lectotype: EKMAN, 3598 S!, holotype: B+.

Stenostomum myrtifolium Griseb. Fl.Br.W.I. 1861: 334.

Holotype: BRACE 445. GOET.

Syn.: Antirhea myrtifolia Urb. Symb. Antill. 1: 440. 1899.

Stenostomum nipense (Borhidi & Muniz) Borhidi & M.Z. Fernandez l.c. 152. 1995.

Basionym: Antirhea nipensis Borhidi & Muñiz Abstracta Bot. Budapest 7: 41. 1977.

Holotype: SV 27798 HAC!

Stenostomum obtusifolium (Urb.) Britt. & Wils. Sci. Surv. Porto Rico 6: 237. 1925.

Basionym: Antirhea obtusifolia Urb., Symb. Antill. 1: 435. 1899.

Holotype: EGGERS 1244 B+. Lectotype: SINTENIS 2569, NY!

Stenostomum occidentale (Urb.) Borhidi & M.Z. Fernandez l.c. 155. 1995.

Basionym: Antirhea occidentalis Urb. Symb. Antill. 9: 529. 1928.

Holotype: EKMAN, 11913 B+. Lectotype: S!

Stenostomum oliganthum (Urb.) Borhidi comb. nova

Basionym: Antirhea oligantha Urb. Symb. Antill. 7: 411. 1912.

Holotype: FUERTES 1471 B+. Lectotype: FUERTES 1496 A!

Stenostomum ophiticola (Alain) Borhidi & M.Z. Fernandez l.c. 153. 1995.

Basionym: Antirhea ophiticola Alain Contr. Ocas. Mus. Hist. Nat. de la Salle, 17: 1. 1959.

Holotype: ACUÑA & ZAYAS 19806 SV/HAC!, isotype: LS/HAC.

Stenostomum orbiculare (Alain) Borhidi & M.Z. Fernandez l.c. 152. 1995.

Basionym: Antirhea orbicularis Alain Contr. Ocas. Mus. Hist. Nat. de la Salle, 17: 2. 1959.

Holotype: CLEMENTE 6219 LS/HAC!, isotype: NY!, US!

Stenostomum pedicellare (Borhidi & Bisse) Borhidi & M.Z. Fernandez l.c. 154. 1995.

Basionym: Antirhea pedicellaris Borhidi & Bisse Acta Bot. Acad. Sci. Hung. 28: 87. 1982.

Holotype: HAJB 17793 Bisse & Lippold, isotype: JE.

Stenostomum pitonianum (Urb. & Ekm.) Borhidi comb. nova

Basionym: Antirhea pitoniana Urb. & Ekm. Ark.f.Bot. 21A No. 5: 82. 1927.

Holotype: EKMAN, H 4596 S!, isotypes: A!, US!

Stenostomum portoricense Britt. & Wils. Sci. Surv. Porto Rico 6: 564. 1930.

Syn.: Antirhea portoricensis Standl. N. Amer. Fl. 32: 368. 1934.

Holotype: BRITTON 9150 NY!

Stenostomum radiatum Griseb. Cat.Pl.Cub. 1866: 132.

Holotype: Wr. 2712. GOET., isotype: GH, MBG!, NY!, S!, US!

Syn.: Antirhea radiata Urb. Symb. Antill. 1: 435. 1899.

Stenostomum pauciflorum Wr. in Sauv. Anal. Acad. Habana 6: 67. 1869.

-- ssp. **radiatum**

-- ssp. **haitiense** (Borhidi) Borhidi comb. nova

Basionym: Antirhea radiata (Griseb.) Urb. ssp. haitiensis Borhidi Acta Bot. Hung. 29: 193. 1983.

Holotype: EKMAN, H 10149 S!

Stenostomum resinosum (Vahl.) Griseb. Fl.Br.W.I. 1861: 334.

Basionym: Laugeria resinosa Vahl. Eclog. 1: 27. 1796.

Syn.: Antirhea resinosa Cook & Coll. Contr. U.S. Nat. Herb. 8: 82. 1903.

Terebraria resinosa Sprague

Neolaugeria resinosa Nicols. Brittonia 31: 121. 1979.

Stenostomum reticulare Borhidi & M.Z. Fernandez Acta Bot. Hung. 38: 147. 1995.

(Syn.: Antirhea reticularis Borhidi & Fernandez in schaedis)

Stenostomum revolutum Borhidi Acta Bot. Hung. 38: 148. 1995.

(Syn.: Antirhea revoluta Borhidi in schaedis)

Stenostomum rotundatum Griseb. Cat. Pl. Cub. 1866: 132.

Holotype: Wr. 2714 GOET.!, isotype: GH, HAC!, MBG!, NY!, S!, US!

Syn.: Antirhea rotundata Hook.f. in Benth. & Hook. Gen.Pl. 2: 100. 1873.

Stenostomum scrobiculatum (Urb.) Borhidi & Fernandez l.c. 154. 1995.

Basionym: Antirhea scrobiculata Urb. Symb. Antill. 9: 529. 1928.

Holotype: EKMAN, 15880 B^+. Lectotype: S!, isolectotype: NY!

Stenostomum shaferi (Urb.) Borhidi & M.Z. Fernandez l.c. 155. 1995.

Basionym: Antirhea shaferi Urb. Feddes. Repert. 13: 479. 1915.

Holotype: SHAFER 8142 B^+. Lectotype: SHAFER 3160 NY!, isolectotypes: HAC!, US!

Stenostomum sintenisii (Urb.) Britt. & Wils. Sci. Surv. Porto Rico. 6: 238. 1925.

Basionym: Antirhea sintenisii Urb. Symb. Antill. 1: 438. 1899.

Holotype: SINTENIS 5945 B+. Lectotype: US!

Stenostomum tomentosum (Sw.) DC. Prodr. 4: 460. 1830.

Basionym: Laugieria tomentosa Sw. Prodr. 1788: 48.

Type: Sw. s.n. S!

Syn.: Antirhea tomentosa Urb. Symb. Antill. 7: 412. 1912.

Stenostomum urbaniarum (C.T. White) Borhidi & M.Z. Fernandez l.c. 155. 1995.

Basionym: Antirhea urbaniana C.T. White J. Arn. Arb. 27: 121. 1946.

Holotype: Wr. 2710 GOET!. Lectotype: GH, HAC!, MBG!, NY!, S!

Syn.: Antirhea tenuiflora Urb. Symb. Antill. 1: 438. 1899. non F. Muell.

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TWO NEW PHIALANTHUS (RUBIACEAE) SPECIES FROM CUBA

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Two new species of the genus Phialanthus Griseb. (Rubiaceae) are described. P. peduncularis is of the Cristal Mts of Oriente Province, while P. bissei is a narrow local endemic of the Isle of Pines (Isle of Youth).

Phialanthus peduncularis Borhidi sp. nova

Arbor parva. Rami veteriores cinereo-nigrescentes, cylindracei, striati, hornotini 4-anguli, resinosi, breviter papilloso-pilosi, internodiis ad ramos 0.5—1 cm, ad ramulos 2—5 mm longis. Stipularum vagina flavo-ferruginea, membranacea, 1—2 mm longa, pilosa, margine fimbriato-ciliata, 4-denticulata, dentibus 2 logioribus apiculatis. Folia 1—3 mm longe petiolata, oblongo-elliptica, oblongo-lanceolata vel lineari-lanceolata, basi longe cuneata, sensim in petiolum protracta, apice acuminata et obtusa, 1—2,2 cm longa et 3—5 mm lata, nervo medio supra per ttotam longitudinem impresso, subtus prominente, lateralibus utrinque nullis, margine anguste recurva, supra opaca, in sicco nigra, subtus pallide flavicanti-viridiuscula, subcoriacea.

Inflorescentiae axillares, pedunculatae, pedunculis 1—4 mm longis suffulta, 5—9-flora; involucrum breviter cupuliforme, margine obsolete-denticulatum et fimbriatum, rariter lobulis 2 linguiformibus fragilibusque caducis suffultum, 1 mm longum, pilosum. Flores pedicellati. Pedicelli 1—3 mm longi, centrales saepe supra medium connati. Flores 4-meri, calycis tubus leviter 4-angulatus, pilosus, inferne breviter attenuatus, lobi oblongo-spathulati, apice rotundati tubo plus-minus aequilongi, 1,5—2 mm longi, 1,2 mm lati, inter lobulos saepe denticulis interjectis. Corolla 2 mm longa, usque ad 2/3 longitudinis coalita, tubus infundibuliformis, lobi ovati, rotundati. Stamina corollae supra basi affixa, longe exserta, filamenta

2—2,5 mm longa antherae ovatae 0,5 mm longae. Stylus 2 mm longus, apice capitatus. Ovula in loculis solitaria, oblonga ab apice loculi pendula.

Holotypus: LOPEZ FIGUEIRAS 201 HAC; Prov. Oriente, Sierra del Cristal, Rio Lebisa, 26.08.1959.

Obs.: Phialantho oblongato Urb. similis, a qua inflorescentiis pedunculatis, floribus pedicellatis involucro atque calycis pilosis certe specifice differt. A Phialantho rigidi Urb. inflorescentiis pedunculatis, floribus pedicellatis, atque foliis supra nitidis facile distinguitur.

Phialanthus rigidus Griseb. **ssp. bissei** ssp. nova

Frutex vel arbor parva, rami teretes, breviter pilosi. Stipulae connatae in annulum truncatum, 1—1.5 mm altum, pilosum. Folia petiolo 1—2 mm longo suffulta, lamina ovata, elliptica vel oblongo elliptica, basi attenuata et obtusa vel breviter rotundata, apice obtusa vel rotundata, brevissime apiculata, 1—4 cm longa et 0.7—1.2 cm lata, nervo medio supra impresso, subtus prominente, lateralibus utrinque nullis, limbus ipse glaber et discolor. Involucrum inflorescentiae infundibuliforme, 1 mm alta et 1—2 mm lata, truncata et margine fimbriata, glabra. Inflorescentiae 4—6-florae, pedicellum exsertum. Hypanthium atque tubus calycis 2 mm longi, oblongo-ovati, pilosi. Lobi calycini 4, anguste spathulati, tubo aequilongi vel breviores. Tubus corollae e basi anilliformi et angustissimo superne valde dilatatus, 2—3 mm longus, lobi corollini late ovati tubo aequilongi, stamina longe exserta. Ovarium biloculare, ovula in loculis solitaria, anguste oblonga.

Holotypus: HAJB 19844, Cuba, Isla de Pinos, Camino entre Cayo Piedras Punda del Este, sobre caliza. Col.: BISSE 24.07.1971. Isotypi: HAC, BP.

Specimine examinata: HAJB 1776, Cuba, Isla de Pinos, Monte quemado hacía Punta del Este. Col.: BISSE, 02.03.1967. — HAJB 12638, Isla de Pinos, entre Cayo Piedras y Punta del Este. Col. BISSE & LIPPOLD febr. 1969. — HAJB 32766, Ibidem, Col. BISSE & al. 22.09.1976.

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A NEW OCCURRENCE OF OROBANCHE NANA NOË ON SZÁRSOMLYÓ IN THE VILLÁNY HILLS

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On Szársomlyó in the Villány Hills a new occurrence of Orobanche nana Noë, which was thought to be extinct, was observed. This paper compares the morphological features of Orobanche nana with those of the very similar Orobanche ramosa L., using data from literature and specimens from herbaria. As an interest in science history and for showing some morphological features the author publishes some photos of herbarial specimens collected by the author herself. The paper also treats the coenological and ecological characteristics of its habitat on Szársomlyó, on the basis of five classical coenological releves, using relative ecological indicator values and social behaviour types of species.

Introduction

In 1990 Orobanche nana, a Mediterranean relic plant species, which was thought to be extinct, has been found again on Szársomlyó in the Villány Hills (Fig. 1).

This species was first collected in Hungary by István NAGY in 1960, but he misdetermined it as Orobanche arenaria (PRISZTER 1966). In 1964 it was collected and determined by PRISZTER (1966) as Orobanche nana, a new species in Hungary. He found the plant on the western part on the hill, not far from the ridge, in a community of Tilio argenteae—Quercetum petreae-cerris. The place of occurrence is shown on the map of SZÁRAZ (SZÁRAZ et al. 1985). Since then this area was destroyed by a querry, so the species was considered as extinct (SOÓ 1980; PRISZTER 1985, 1987; SZÁRAZ et al. 1985). HORVÁT (1977) reports two more occurrences: from Alsónána (Szekszárd Hills) and, referring to KÁROLYI, from Tubes Hill (Mecsek Hills); but these data are probably due to misprint.[1]

[1]PRISZTER (1985) considers O. nana as a synonym of O. oxyloba. Others (TUTIN et al. 1972; UHLICH 1992) consider both as individual species.

Fig. 1. Orobanche nana Noë
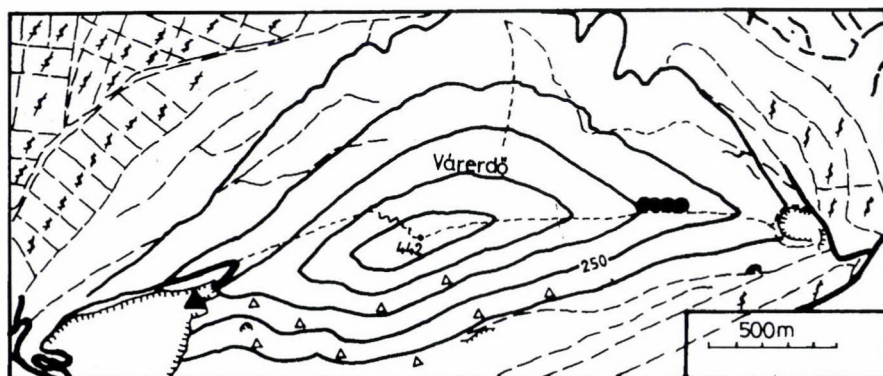
Fig. 2. Occurrence of O. nana on Szársomlyó.
Δ: extinct population, •: present population

Fig. 3. O. nana and its host plant Lamium maculatum

I found the plant on the eastern slope of the hill. Its place of occurrence is situated north of the ridge, in a 15-20 m wide strip of the edge of Várerdő. Examining the forest along the ridge, 20-25 individuals were found. According to my examinations up to now Orobanche nana lives only in this narrow strip (Fig. 2). Since then I was able to observe its flowering individuals every year from middle of July to end of August. Its host plant was found to be Lamium maculatum (Fig. 3).

Morphological description of Orobanche nana

Since distinguishing O. nana and O. ramosa is not easy, TUTIN et al. (1972) treat O. nana as subspecies of O. ramosa: O. ramosa L. ssp. nana (Reuter) Coutinho, I compared the characteristic features of my collected plants to herbarium specimens of both above-mentioned species.

Figs 4—5. Specimens collected by Noë in the 1800s

The examined specimens of O. nana originate from Mediterranean areas, some of them are collected by the author, NOË (Figs 4—7). I have not been able to found the specimens collected by PRISZTER and NAGY on Szársomlyó. My results are summarized in Table 1. The characteristics of the herbarium specimens are not always identical with literature, and there are size overlaps between the two species. Looking for further differences I compared scanning EM pictures of the seeds of the two related species (Figs 8—10). On the surfaces of the seeds I cannot detect any differences, but there is a significant difference between the sizes of the seeds of the two species.

The plants collected on Szársomlyó, according to decisive majority of their characteristics, are proved to be Orobanche nana. They can be found in the Herbarium of Janus Pannonius Museum, Pécs.
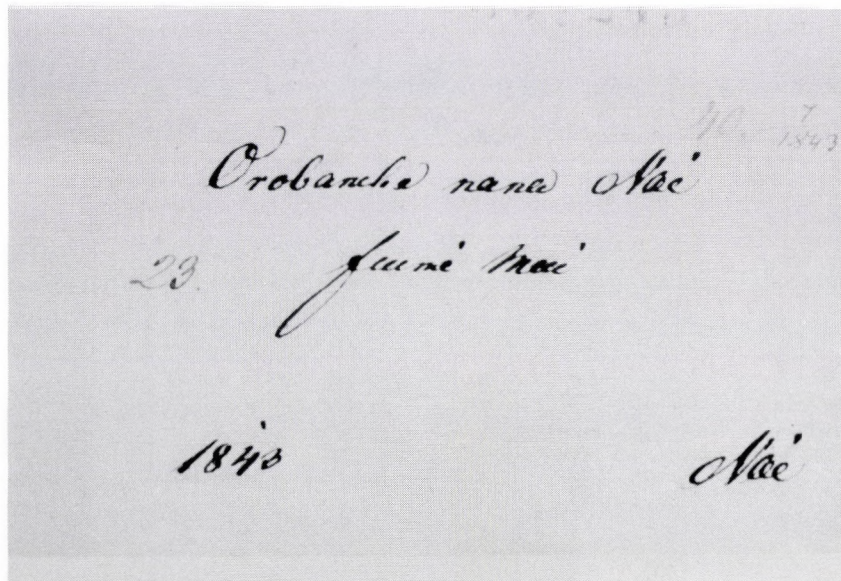
Fig. 6. Herbarium card of Noë

Description of the habitat

The habitat, regarding its situation, microclimate and species composition, is particular. It lays pretty close to the ridge, so it is warmer than the lower-situated parts of the northern slope, but cooler and more humid than the southern slope. The coenological place of the community living here is debated: LEHMANN (1975) names it karstic shrubforest, BORHIDI (1991) Rusco-Ornetum.

For more precise characterisation of the habitat I have made coenological relevès, whose results are summarized in Table 2. Coenological characteristics, social behaviour type and naturalness indicator values of the species were determined according to BORHIDI (1993), life forms and floral element types according to SOÓ (1968—80). All the data where calculated on the basis of group percentages; the results are presented graphically (Figs 11—20).

The community is situated on the northern part of the ridge, 250-300 m a.s.l., well bordered from the Asperulo taurinae-Carpinetum community of the northern slope. The closed canopy layer is dominated by individuals of Fraxinus ornus of the height of 7-8 m, with thick trunks (20-25 cm trunk

Fig. 7. Specimens collected in Italy

diameter), accompanied by *Acer campestre*, *Ulmus minor* and a few *Quercus pubescens*. In the herb layer there are *Quercetalia-pubescentis-petreae* species (15.31%), *Orno-Ostryon* species (5.86%), and *Fagetalia* elements too. Dominant and constant species are: *Lamium maculatum*, *Fragaria viridis*, *Geum urbanum*, *Helleborus odorus*. In the early spring aspect dominate *Ficaria verna*, *Corydalis solida*, *Corydalis cava*. Subconstant species: *Brachypodium silvaticum*, *Campanula persicifolia*, *Ornithogalum sphaerocarpum*, *Ficaria verna*, *Galanthus nivalis*, *Iris variegata*. Considering the social behaviour types, there are generalists (G: 43.24%) and competitors (C: 17%) in the greatest proportion; the proportion of natural disturbance tolerants is high too (DT: 21.17%). Naturalness indicator value of the area is 3.5.

Considering relative ecological indicator values the occurring species represent a habitat semihumid, moderately rich in nutrients, half-shadowed

Table I

Morphological characteristics of Orobanche nana and O. ramosa based on literature and herbarial specimens

		Orobanche ramosa	Orobanche nana	Plants found on Szársomlyó
stem	1	branching	simple, seldom branching	
	2	66% branching 34% simple	88% simple 12% branching (Figs 4–8)	simple, 1-2 plants with 1-2 short branches
height (cm)	1	(5-) 10-30 (-40)	5-10 (-30)	
	2	8-32	4.5-16.5 70% under 10	6-10
length of corolla (mm)	1	10-12 (17)	11-15	
	2	7-19	12-17	9-14
colour of corolla	1	pale blue or violet (seldom tawny)	blue or violet; tip of corolla lobes are darker blue (corolla is seldom white or yellow)	
	2	not visible	not visible	blue; tips of corolla lobes are darker, often bright blue
lip of corolla	1	lobes of lower lip are blunt	lobes of lower lip are pointed (seldom blunt)	
	2	on herbarial specimens is not clearly distinguishable		corolla lobes are a little pointed
number of flowers	1	many	5-10 (-14) few	
	2	2-34	4-20	2-10
calyx	1	teeth of calyx are shorter than tube of calyx; they are pointed lance-shaped triangular	teeth of calyx are as long or longer than tube of calyx; they are filamental awl-shaped	
	2	material was so crumbled that it was no use to measure the teeth of calyx		teeth of calyx are more or less fila-mental, as long as tube of calyx or a little longer
anthers	1	not hairy	not hairy	
	2			usually 3-4 long hairs are visible on it
seed (mm)	1	0.4	0.27-0.33	
	2	0-25-0.27 (Fig. 8)	0.13-0.16 (Fig. 9)	0.16-0.19 (Fig. 10)

Table 1 (cont.)

		Orobanche ramosa	Orobanche nana	Plants found on Szársomlyó
host	1	usually domesticated plants (Nicotiana, Helianthus, Cannabis etc.) but various wild plants too	various wild plants (Fabaceae, Labiatae, Veronica, Torilis Capsella)	not known
	2	in 90% domesticated plants + Melilotus officinalis, Trifolium alexandrii, Lamium maculatum, Humulus lupulus, Cirsium arvense, Alliaria petiolata	Melilocus neopolitana, Vicia angustifolia, Coronilla scorpionis	Lamium maculatum
number of plants examinated		125	53	15
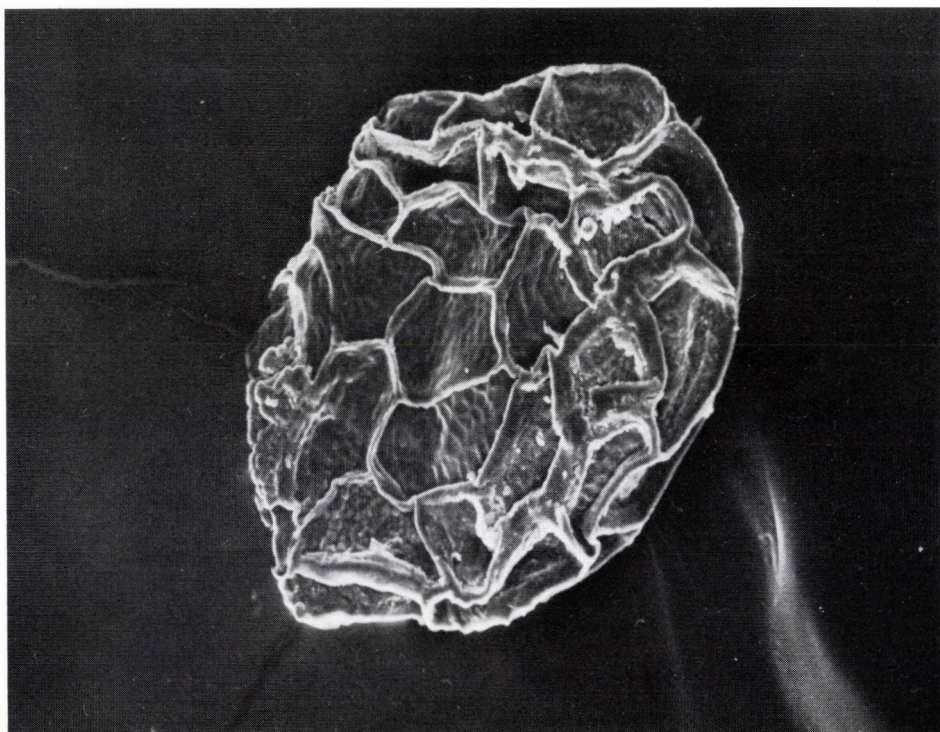
Fig. 8. Seed of O. ramosa (x420)
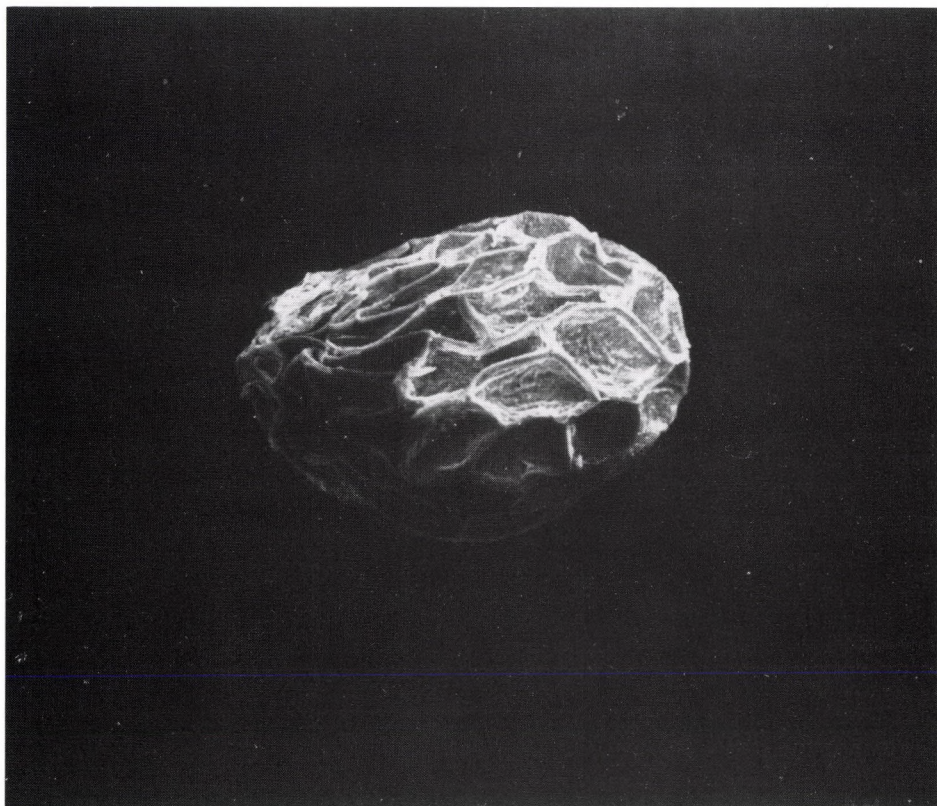
Fig. 9. Seed of O. nana (x420)

and half-light. Considering the temperature indicator values, most of the occurring species show a thermoclinate characteristic for submontane broad-leaved forest belt, thermophilous forest and forest-steppe belt.

The habitat, from ecological and also from coenological viewpoint, is transitionary.
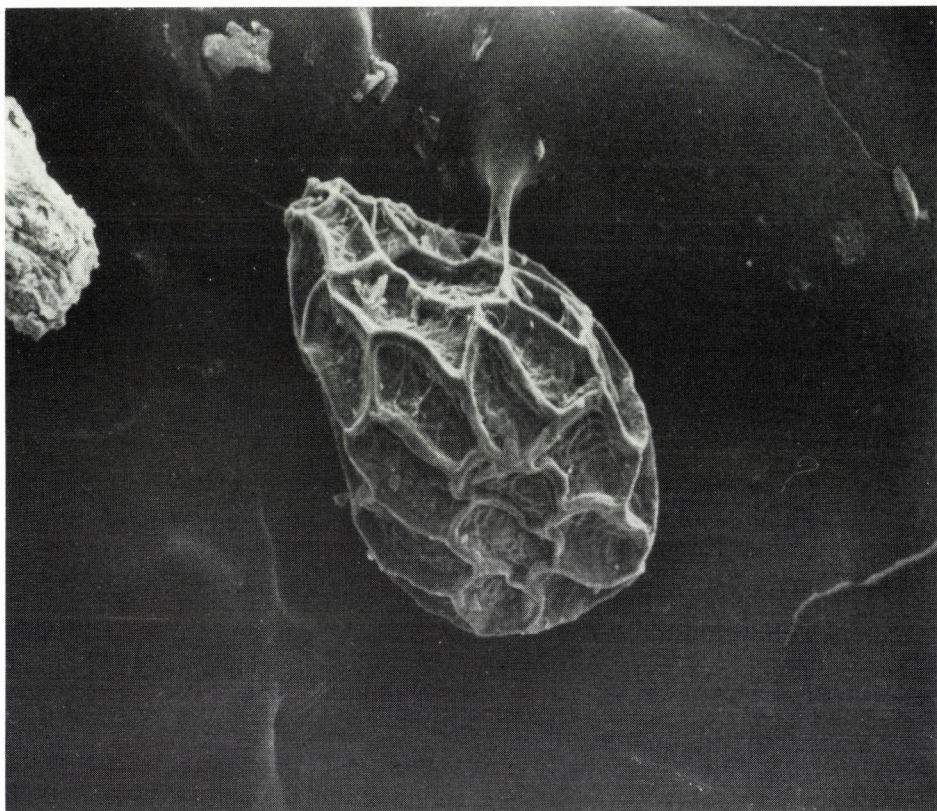
Fig. 10. Seed of plants collected on Szársomlyó (x420)

Table 2

Coenological releves made in the habitat of O. nana

	1	2	3	4	5	A—D	K
Canopy layer							
height (m):	7.5	7	8	8	9		
cover (%):	75	60	60	70	70		
Orno-Ostryon:							
Fraxinus ornus	60	30	25	35	56	25—60	V
Quercetalia pubescentis-petraeae:							
Quercus pubescens	7	10	0	0	12	7—12	III
Fagetalia:							
Acer platanoides	0	0	0	0	1.5	1.5	I
Querco-Fagetea:							
Acer campestre	8	20	30	35	1.5	1.5—35	V
Ulmus minor	0.1	5	5	0.1	0	0.1—5	IV
Shrub layer							
height (m):	2	4	3	5	4.5		
cover (%):	40	40	35	35	47		
Orno-Ostryon:							
Fraxinus ornus	0	0	0.1	15	15	0.1—15	III
Quercetalia pubescentis-petraeae:							
Cornus mas	20	20	2.5	15	25	2.5—25	V
Crataegus monogyna	0.1	0.1	0.1	0.1	0.1	0.1	V
Euonymus verrucosus	10	0	0	0	0.1	0.1—10	II
Quercus pubescens	0	0.1	0	0	0	0.1	I
Querco-Fagetea:							
Acer campestre	0.1	0.1	0.1	2.5	0.1	0.1—2.5	V
Ulmus minor	0.1	0.1	2.5	0.1	0	0.1—2.5	IV
Ligustrum vulgare	0	0	0.1	0	0	0.1	I
Ruscus aculeatus	0	0	0.1	0	0	0.1	I
Tilia argentea	0.1	0	0	0	0	0.1	I
Tilia platyphyllos	0	0	0	0	0.1	0.1	I
Prunetalia spinosae:							
Rosa canina	10	15	25	5	0.1	0.1—25	V
Prunus spinosa	0	0	0	0.1	0.1	0.1	II
Herb layer							
cover (%):	70	70	65	40	85		
Orno-Ostryon:							
Orobanche nana	0.1	0.1	0.1	0	0	0.1	III
Fraxinus ornus	0	0.1	0	0.1	0	0.1	II

Table 2 (cont.)

	1	2	3	4	5	A—D	K
Geranion sanguinei:							
Fragaria viridis	0.1	0.1	5	2	1	0.1—5	V
Iris variegata	0	0.1	1	0.1	0.1	0.1—1	IV
Quercetalia pubescentis-petraeae:							
Gagea pratensis	0.1	0.1	0.1	0.1	0.1	0.1	V
Sedum maximum	0.1	0.1	0	0.1	0.1	0.1	IV
Campanula bononiensis	0.1	0	0	0	0.1	0.1	II
Cornus mas	0.1	0.1	0	0	0	0.1	II
Euonymus verrucosus	0.1	0.1	0	0	0	0.1	II
Euphorbia polychroma	0.1	0	0	0	0	0.1	I
Lithospermum purp.-coer.	0	0	0	0	2	2	I
Polygonatum odoratum	0	0	0	0	20	20	I
Fagetalia:							
Corydalis cava	5	0.1	0.1	0.1	0.1	0.1—5	V
Galanthus nivalis	0	5	1	10	8	1—10	V
Ficaria verna	0	15	38	5	5	5—38	IV
Scilla vindobonensis	0	3	1	1	0	0.1—3	III
Arum maculatum	0	0	0	0.1	0.1	0.1	II
Acer platanoides	0	0	0	0	0.1	0.1	I
Euphorbia amygdaloides	0	0.1	0	0	0	0.1	I
Pulmonaria officinalis	0.1	0	0	0	0	0.1	I
Querco-Fagetea:							
Anemone ranunculoides	0.1	0.1	0.1	0.1	0.1	0.1	V
Corydalis solida	15	25	0.1	0.1	5	0.1—25	V
Helleborus odorus	3	2.5	2	3	1	1—3	V
Viola odorata	2.5	3	5	1	7	1—7	V
Ruscus aculeatus	4	0	0.1	5	0	0.1—5	III
Acer campestre	0	0.1	0.1	0	0	0.1	II
Hedera helix	0	0.1	0	0	0.1	0.1	II
Ligustrum vulgare	1	0	0.1	0	0	0.1—1	II
Tilia argentea	0	0.1	0	0	0	0.1	I
Ulmus minor	0	0	0	1	0	1	I
Prunetalia spinosae:							
Rosa canina	0.1	0.1	1	0.1	1	0.1—1	V
Crataegus monogyna	0.1	0	0	0.1	1	0.1—1	III
Festuco-Brometea and **Festucetalia valesiacae:**							
Ornithogalum spaeroceph.	1	0	1	0.1	0.1	0.1—1	IV
Festuca rupicola	25	0.1	6	0	0	0.1—25	III
Teucrium chamaedrys	3	0	0	0.1	0	0.1—3	II
Asparagus officinalis	0	1	0	0	0	1	I
Festuca valesiaca	0.1	0	0	0	0	0.1	I
Melica ciliata	0	0	0	0.1	0	0.1	I
Veronica spicata	0	0	0	0	0.1	0.1	I
Other species:							
Antriscus cerefolium	5	0	1	0.1	2	0.1—5	V

Table 2 (cont.)

	1	2	3	4	5	A—D	K
Geum urbanum	5	0	1	0.1	2	1—25	V
Lamium maculatum	37	35	7	15	40	7—40	V
Veronica hederifolia	2	5	1	0.1	1	0.1—5	V
Alliaria petiolata	1	1	0.1	0.1	0	0.1—1	IV
Brachypodium silvaticum	0.1	0.1	0.1	0.1	0	0.1	IV
Geranium molle	0.1	0.1	0.1	0.1	0	0.1	IV
Antriscus sylvestris	1	0	0.1	0.1	0	0.1—1	III
Bilderdykia convolvulus	1	0.1	0	0.1	0	0.1—1	III
Lamium purpureum	0.1	0.1	0.1	0	0	0.1	III
Calepina irregularis	0.1	0	0	0.1	0	0.1	II
Galium aparine	0.1	0.1	0	0	0	0.1	II
Veronica chamaedrys	0	0.1	0	0	2	0.1—2	II
Vicia hirsuta	0.1	0.1	0	0	0	0.1	II
Vicia sepium	0.1	0.1	0	0	0	0.1	II
Achillea collina	0	0	0	0.1	0	0.1	I
Ajuga reptans	0	0.1	0	0	0	0.1	I
Berteroa incana	0.1	0.1	0	0	0.1	0.1	I
Bromus sterilis	0.1	0	0	0	0	0.1	I
Bromus tectorum	0	0	0	0	0.1	0.1	I
Chelidonium majus	0	0	0	0	0.1	0.1	I
Eryngium campestre	0	0	0	0.1	0	0.1	I
Geranium columbinum	0.1	0	0	0	0	0.1	I
Plantago lanceolata	0.1	0	0	0	0	0.1	I
Taraxacum officinale	0	0	0	0.1	0	0.1	I

Exposition:	E	E	E	E	E
Inclination:	15	15	15	20	20
Height a.s.l. (m):	250	260	260	280	280
Rocks (%):	3	0	0	1	0
Fallen leaves and bare (%):	10	30	35	50	15
Size of relevè (m^2):	100	100	100	100	100
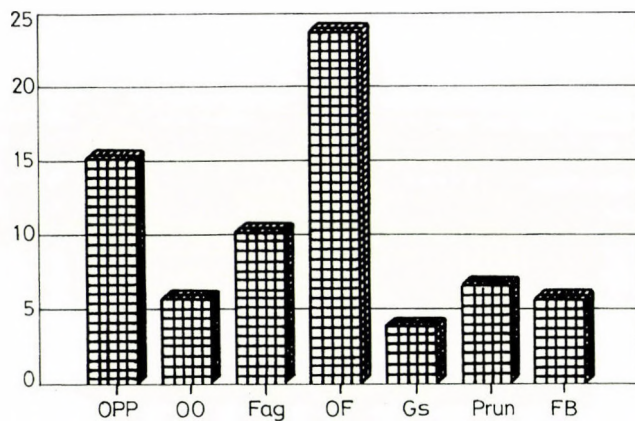
Fig. 11. Percentage of character species.
Qpp: Quercetalia pubescentis-petraeae, OO: Orno-Ostryon, Fag: Fagetalia, QF: Querco-Fagetea, Gs: Geranion sanguinei, Prun: Prunetalia spinosae, FB: Festuco-Bometea and Festucetalia valesiacae
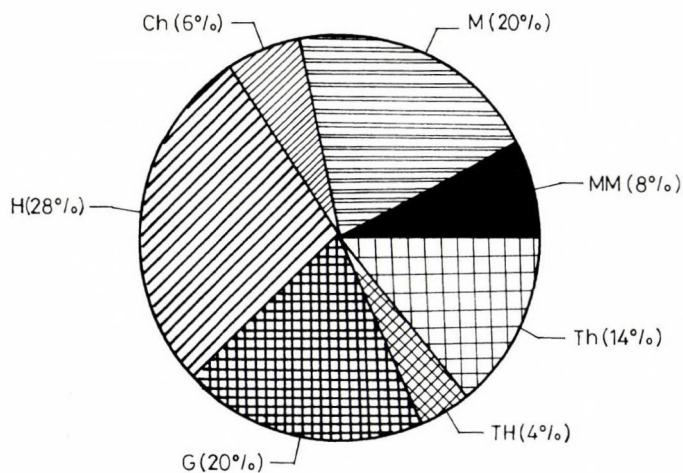
Fig. 12. Percentage of life form elements
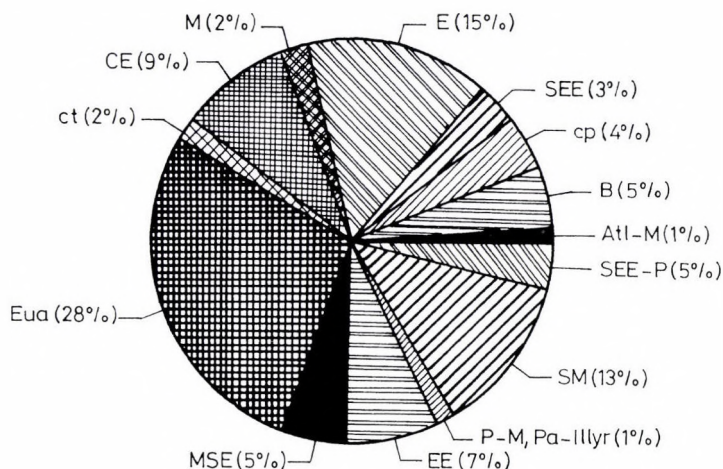
Fig. 13. Percentage of floral elements.
Atl: Atlantic-Mediterranean, B: Balcanic, CE: Central-European, Cp: circumpolar, SEE: South-East-European, E: European, EE: East-European, Eua: Eurasian, ct: continental, MSE: Middle-South-European, M: Mediterranean, P: Pontian, Pa: Pannonian, SM: submediterranean
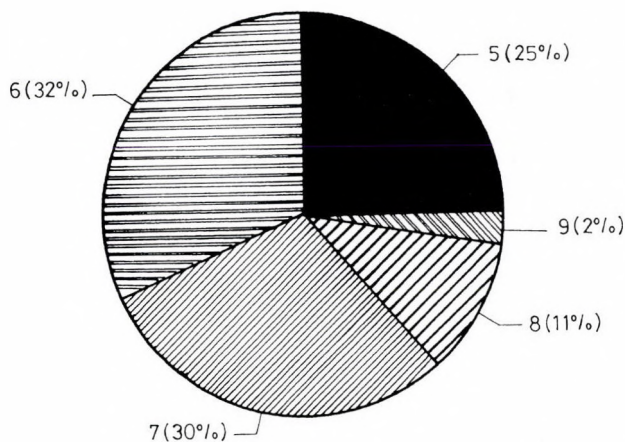
Fig. 14. The relative "temperature figures" (TB).
4: montane needle-leaved forest or taiga belt, 5: montane mesophilous broad-leaved forest belt, 6: submontane mesophilous broad-leaved forest belt, 7: thermophilous forest or woodland belt, 8: submediterranean woodland and grassland belt, 9: eumediterranean evergreen belt
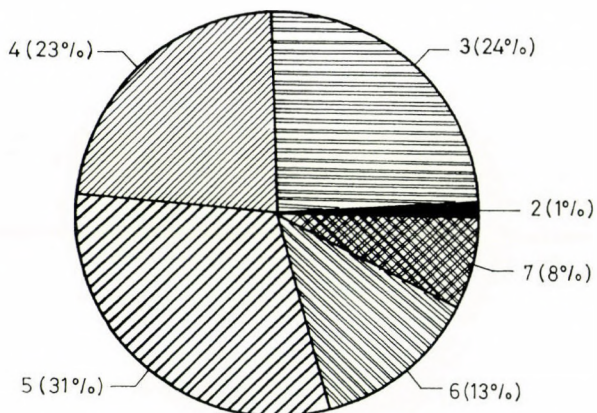
Fig. 15. The relative "moisture figures" (WB).
1: plants of extremely dry habitats or bare rocks, 2: xero-indicators on habitats with long dry period, 3: xero-tolerants, but eventually occurring on fresh soils, 4: plants of semidry habitats, 5: plants of semihumid habitats, under intermediate conditions, 6: plants of fresh soils, 7: plants of moist soils not drying out and well aerated
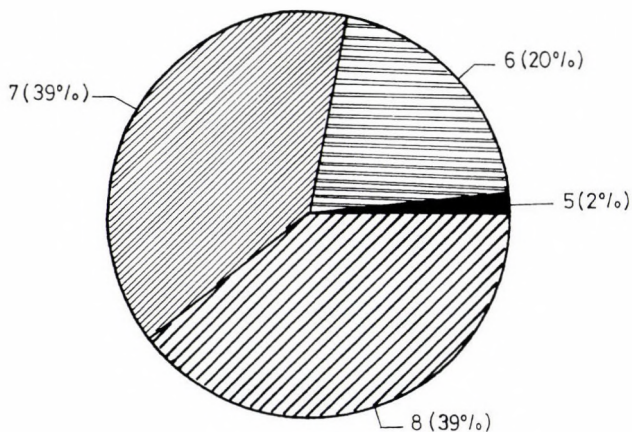
Fig. 16. Reaction figures (RB).
4: moderately acidphilous plants, 5: plants of slightly acid soils, 6: mostly on neutral soils but also in acid ones, generally widely tolerant, more or less indifferent plants, 7: basi-frequent plants, mostly on basic soils, 8: basiphilous plants, 9: explicitly calciphilous plants and ultrabasic specialists
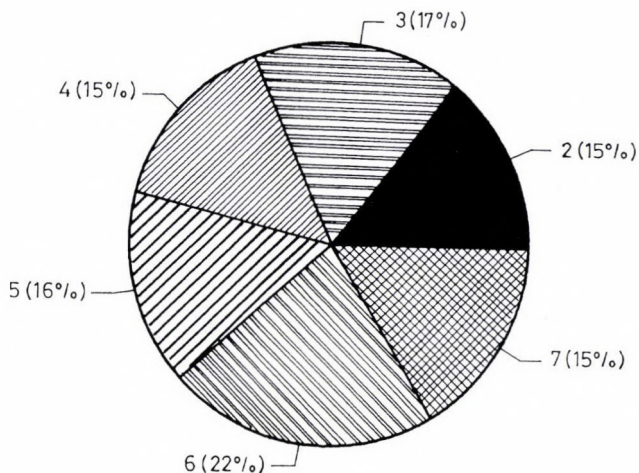
Fig. 17. Nitrogen figures (NB).
1: only in soils extremely poor in mineral nitrogen, e.g. peat bog plants, 2: plants of habitats very poor in nitrogen, 3: plants of moderately oligotrophic habitats, 4: plants of sub mesotrophic habitats, 5: plants of mesotrophic habitats, 6: plants of moderately nutrient rich habitats, 7: plants of soils rich in mineral nitrogen, 8: N-indicator plants of fertilized soils, 9: plants only on hyperfertilized soil, extremely rich in mineral nitrogen (indicating pollution, manure deposition)
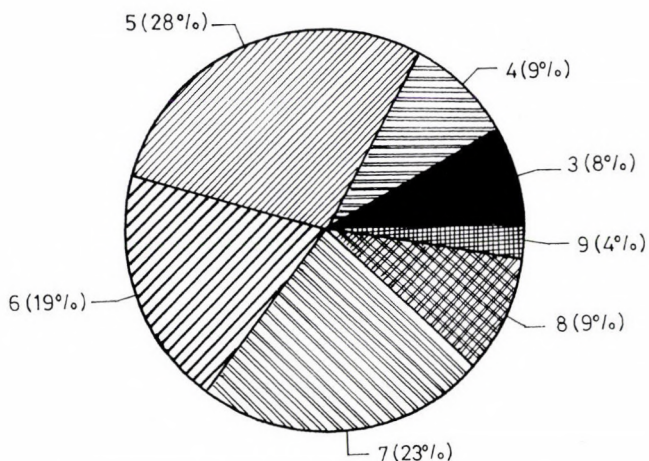
Fig. 18. "Light figures" (LB).
3: shadow plants, 4: shadow-half-shadow plants, 5: half-shadow plants, 6: half-shadow-half-light plants, 7: half-light plants, 8: light plants, 9: full light plants
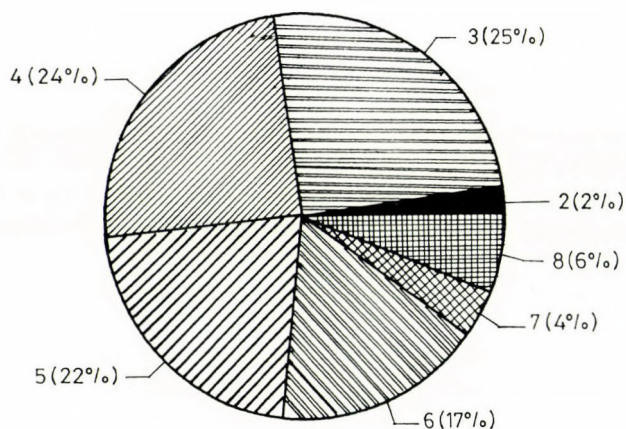
Fig. 19. "Continentally figures" (CB).
2: oceanic species, mainly in West Europe and western Central Europe, 3: oceanic-suboceanic species, area in whole Central Europe, 4: suboceanic species, mainly in Central Europe but reaching to East, 5: intermediate type with slight suboceanic-subcontinental character, 6. subcontinental, main area eastern, Central Europe, 7: continental-subcontinental species main area in East Europe, 8: continental species reaching only eastern part of Central Europe
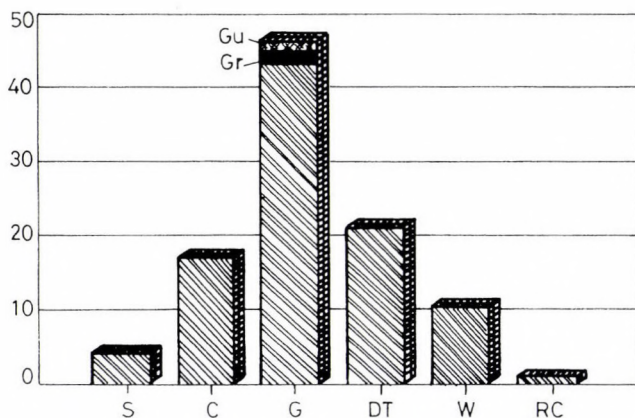
Fig. 20. The Social Behaviour Types of natural habitats (SBT).
S: specialists, C: competitors, G: generalists, Gu: unique generalist, Gr: rare generalist, DT: disturbance tolerants, W: weeds, RC: ruderal competitors

Acknowledgements

I wish to thank for everybody, who have helped me during my work: Dr. Attila Borhidi, Dr. Szaniszló Priszter, Dr. Tibor Simon and Balázs Kevey for professional help, Dr. Borbála Maráz for translating descriptions from Latin; further for the Biology Department Group of ELTE for making the scanning EM photos.

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LOBELIA PRAETERVISA BORHIDI SPEC. NOVA EN LA FLORA DE ESPAÑOLA

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(Llegado: 15 de Agosto, 1992)

In the duplicate materials of the EKMAN's collection received for exchange in the JPU, during its taxonomic revision a sheet appeared under the collection number H 1451 of EKMAN determined as Lobelia rotundifolia Juss. This collection number has not been mentioned in any publication related to the Hispaniolan Lobelias, neither in the publications of URBAN, nor in the great monographic work of WIMMER in Das Pflanzenreich. The specimen turned to represent an undescribed species named by the author as Lobelia praetervisa.

Introducción

En el material duplicado de EKMAN recibido en intercambio de S, durante su revisión, apareció un ejemplar con el número de colecta H 1451 de EKMAN, determinado como Lobelia rotundifolia Juss. Después de haber revisado la literatura constatamos, que este número de colecta no se haya publicado ni en las publicaciones numerosas de URBAN (1899, 1920, 1926), ni en la monografía grande de WIMMER (1953), ni por otros autores, que trabajaban en este grupo taxonómico, como O. C. SCHMIDT (1933) y McVAUGH (1943), es decir, este ejemplar no ha sido taxonómicamente revisado. Nuestros estudios concluyeron en el resultado, que el ejemplar cuestionado no es idéntico con la L. rotundifolia Juss., sino representa un taxon todavía no descrito. Hay que admitir, que la L. rotundifolia es una especie bastante conflictiva, porque la planta tiene una gran variabilidad en cuanto a la forma de las hojas (URBAN 1900: 455) y casi no hay ejemplares, que cumplan los criterios caracteristicos que contiene la descripción (JUSSIEU in A. DE CANDOLLE Prodr. VII (2): 383—384) de esta espécie (WIMMER 1953: 286).

El ejemplar H 1451 de EKMAN tiene hojas mas redondeadas que muchos ejemplares de la L. rotundifolia, peró en otros caracteres no coincide con la descripción de DE CANDOLLE, teniendo hojas carnosas, setuloso-hirsutas,

tallo escabroso-estriado, a veces ramificado, cáliz y corolla setuloso-pubescentes. Además de estos, hay todavia un caracter interesante de esta planta, que es el pedicelo rígido, persistente, que depués de la caida de la flor se mantiene como espina encorvada por arriba. Consideramos, que el ejemplar de número H 1451 de EKMAN con sus caracteres enumerados arriba representa una especie nueva, posiblemente endémica de la Sierra de la Selle en Haiti, que nombramos como Lobelia praetervisa, indicando la inadvertencia cometida en la clasificación de la planta.

Lobelia praetervisa Borhidi **spec. nova**

Frutex basi ramificatus, rami teretes, scabroso-pilosi, longitrorse prominulo-lineati, lenticellosi, superne 4-anguli et breviter sericeo-hirsuti, dense foliosi. Folia crasse carnosa in sicco rugulosa, elliptica vel late obovata, antice rotundata vel truncata, apice ipso brevissime apiculato et reflexo-cuspidato, basi longe attenuata et acuta, in petiolum 3-8 mm longum basi dense hirsutum angustata, 2,5—4,5 cm longa et 1,2—2,5 cm lata, margine leviter incrassata et glanduloso-denticulata, nervo medio utrinque applanato, superne obsoleto, lateralibus apicem versus arcuatis, plerumque inconspicuis; lamina supra viridis, subtus pallida, utrinque pilis rigidis adpresse setoso-hirsuta, supra prominenter calloso-punctata, subtus pilis delapsis impresse punctulata.

Flores in racemo multifloro 15—20 cm longo, rhachi sericeo-hirsuto. Bracteae lineari-lanceolatae, 5—10 mm longae et 1—3 mm latae, inferne sensim majores, euphylloideae, ellipticae. Pedicelli 1—2 cm longi, arcuato-erecti, hirsuti, sub medio minute bibracteolati, floribus delapsis rigidi et glabri, spiniformiter persistentes.

Hypanthium late turbinatum, lobi calycini e basi lata, anguste triangulares vel lineares, hirsuti, 2 inferiores breviores 4—5 mm longi et apice obtusi, 3 superiores 5—6 mm longi, acuminati et acuti, plerumque omnes margine denticulati. Corolla purpurea vel coccinea, 3—4 cm longa, pubescens, lobi superiores 10—15 mm, inferiores 7—12 mm longi, leviter falcati. Stamina glabra, filamenta 20—26 mm longa, antherarum tubus niger, 4—5 mm longus, antherae 2 inferiores barbatae; stylus apice bilobatus, lobi orbiculares, inaequales, dorso villosi.

Holotypus: EKMAN H 1451. Haiti, Massif de la Selle, Morne de la Visite, in low deciduous forest along the path towards Salron, c. 2100 m. Typus: JPU!, isotypus: S. (non vidi).

Obs.: Lobeliae rotundifoliae Juss. affinis et cum illa confundata, sed ab ea foliis carnosis, setoso-hirsutis, inflorescentiis duplo longioribus et hirsutis, corolla pubescenti, pedicellis rigidis, arcuato-erectis, persistentibus atque spinosiformibus, tubo antherarum breviore satis bene differt.

Agradecimientos

El trabajo fue realizado dentro del proyecto Flora Macroantillana y apoyado por el Fondo Científico Nacional de Hungría (OTKA) no. 1299.

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UNA NUEVA PALMA EN CUBA

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(Llegado: Septiembre 30, 1994)

Una nueva palma del género Coccothrinax ha sido descubierto por el J. A. HERNANDEZ cerca de la Costa Sur de oriente, al Oeste de la Base Naval de Guantánamo. La palma nueva — C. pumila Borhidi & J. A. Hernandez —, carece de troncos y forma colonias enanas achaparradas, con hojas plateadas en ambas caras y cerosas en el haz.

Coccothrinax pumila Borhidi & J. A. Hernandez **sp. nova**

Palma nana, gregaria, usque ad 60—70 cm alta. Caules e basi pluries abeuntes tenues cylindracei, 10—25 cm longi, 6—8 cm crassi in diametro. Vagina frondis 6—8 cm longa, 10—12 cm lata, fibris 0.5—1 mm latis laxe intertexta, fibrilloso-tomentosa, pars libera truncata 11—12 cm longa, fibris tomentosis in apices 3—4 flexuosi excurrentibus. Petiolum sine vagina 35—45 cm longum, sub apice 1 cm latum, utrinque convexum, flavo-brunneum, nitidum, apice ligulis obliquis binis suffultum. Ligula adaxialis late triangulari-ovata, apice longe attenuata et mucronata, 1,2 cm longa, contraligula truncata, 2—3 mm longa, apice acutiuscula. Lamina frondis 2/3-orbicularis, basi rotundata vel subcordata, 35—40 cm longa et 30—40 cm lata, segmentis 20—25 praedita, segmenta centralia 35—38 cm longa, 3 cm lata, basi usque ad 6—9 cm longe connata, superne sensim angustata et in apices 13—15 cm longos excurrentia, omnia supra alba, cerifera et nitidula, nervis primariis, secundariis atque tertiariis conspicuis et leviter prominulis suffulta, subtus indumento argenteo fibroso-piloso, atque punctis albis sub indumento prominulis praedita.

Inflorescentiae terminales erectae sed altitudinem foliorum non superantes, e racemis partialibus 2 compositae, 40—50 cm longae, pedunculo 30—32 cm longo, bracteae lanceolatae 8—10 cm longae, rigidae, apice tomentosae. Inflorescentiae partiales 20—25 cm longae, simplice ramificatae,

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ramulis 9—11 suffultae. Flores 2—3 mm longe pedicellati, lobi perianthii 6, 1—1.5 mm longi, basi breviter connati, late triangulares, basi filamentis latiores, Stamina 9, filamenta linearia, lobi perianthii paullo superantia. Fructus globosus, 8—9 mm in diametro, pericarpium papyraceum, punctis prominulis dense dispositis rugulosum. Semen osseum, 6—7 mm in diametro, globosum, sulcis 5 usque ad base directis sulcatum.

Holotypus: HAC sine numero. Oriente, Prov. Guantánamo, Morrillo Chico, area de Hatibonico. Oeste de la Base Naval de Guántanamo. Col.: Juan Antonio Hernandez Valdés, Mayo, 1994.

Obs.: Taxon proximum est Coccothrinax pseudorigida León var. acaulis León a qua species nostra foliis supra albis, cera indutis, segmentis folii planis, punctis vaginae flexuosis tenuibusque facillime distinguitur.

Esta palma pertenece al la sección Longispadiceae, subsección Pauciramosae, donde hay que colocar en la vecinidad del Coccothrinax pseudorigida León, que vive en las sabanas serpentinosas de Camagüey y tiene una variedad sin tronco (var. acaulis León). La nueva especie se distingue de aquello por tener la vaina finamente entretejida con puntas delgadas, flexibles, las hojas plateadas en ambas caras, con un indumento blanco de cera en el haz y con un indumento fibroso blanco en el envés, mientras el Coccothrinax pseudorigida tiene una vaina gruesa, con puntas espinosas de fibras leñosas de hasta 6 cm de largo y 5 mm de ancho además sus hojas son verdes en el haz sin una capa de cera y glabras en el envés. El nombre de la palma nueva Coccothrinax pumila se refiere al tamaño enano de la palma, teniendo troncos de 10—25 cm de alto, y formando colonias achaparradas de hasta 1 m de altura. Crece en matorrales sabanosos subcosteros cerca de Morrillo Chico, sobre caliza gravellosa y arenosa, en la zona de Hatibonico, al Oeste de la Base Naval de Guantánamo.

Agradecimientos

Los autores expresan sus agradecimientos al director del Instituto de Ecología y Sistemática de la Academia de Ciencias de Cuba y al custode del Herbario HAC por asegurar las condiciones del trabajo, que fue realizado dentro del marco del convenio bilateral científico firmado por las Academias de Ciencias de Cuba y de Hungría respectivamente. Los estudios fueron apoyados financiamente también por la Fundación Científica Nacional del Hungría (OTKA No. 1299 y 976 respectivamente).

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THERMO-OSMOSIS: DOES IT HAPPEN TO LIVING SYSTEMS?

I. AN OVERWIEW.

SINGLE CELLS: EGG. PARAMAECIUM. RED BLOOD CELL

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Recent publications refer to the possible role of thermo-osmosis in living systems but they forget to mention some former results. A short review of them is given. Our experimental results published in this paper witness: 1. Hypo-osmotic liquid is transported from a warm part to the cold one in the gel system of an egg. 2. The increase of the chemical thermo-genesis enhances the frequency of pulsation of contractile vacuoles that control the osmotic processes in the Paramaecium. 3. High intensity of light or ultrasound irradiation causes a reversible decrease of the volume of red blood cells. All these phenomena are the result of thermo-osmosis.

Introduction

Recently quite a few new publications referred to the biological role of the thermo-osmosis. Some authors (SHUKLA 1984; GAETA et al. 1987; MITA et al. 1990; ANTONOV et al. 1990) consider the possibility of various ways of transport -- the thermo-osmosis included -- brought about by thermal gradients in living systems. A lot of model experiment of the thermo-osmosis give indirect evidences of this possibility (TASAKA et al. 1984; MENGUAL et al. 1986; TASAKA 1986; TASAKA and FUTAMURA 1986; MENGUAL et al. 1988; MENGUAL and GARCIA-LOPEZ 1988; FERNANDEZ-PINEDA and VAZQUEZ-GONZALEZ 1988; SAKAI et al. 1988; FERNANDEZ-PINEDA and VAZQUEZ-GONZALEZ 1989; ORTIZ-ZARATE et al. 1989; ORTIZ-ZARATE et al. 1990; TANAKA et al. 1990; ORTIZ-TANAKA et al. 1991; GAETA et al. 1991; TAKEYAMA and NAKASHIMA 1991; ORTIZ-ZARATE et al. 1992; TASAKA et al. 1992a, 1992b).[1] However, as far as I know, there is

[1]Small differences of temperature may disturb the measurement of the water potential (WULLSCHLEGER et al. 1988) and of the water transport in plants (O'TOOLE and TOMAR 1982). The temperature gradient has an influence on water transport of the soil, too (NASSAR and HORTON 1989).

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no direct experimental test that bears witness to the significant role of the thermo-osmosis in normal physiological processes. On the other hand, some recent papers bring the possibility into the mind that certain effects could be the result of thermo-osmosis (MOU and LIN 1985; NELSON 1986; SCHRÖDER et al. 1986; HANNA and SCHERER 1986; GLASHEEN and HAAN 1989; IKEDA and TSUKUDA 1989; JEJE and KOOn 1989; PICKARD 1989; RUBINSKY and ETO 1989; ZHU et al. 1989; DAVISKAS et al. 1990; WHEATLEY et al. 1991; ZHU and BECK 1991; JONES et al. 1992; WERNER et al. 1992).

Others do not cite a part of former publications about this subject (e.g. HRYB 1981; GAETA et al. 1987; MITA et al. 1988; ANTONOV et al. 1990; TASAKA et al. 1990). Therefore, we cite these neglected publications and now we report our experiments we published earlier in Hungarian only. This time experiments performed on single cells (eggs, paramecia, red blood cells) are summarized, a second paper will deal with the results of experiments on various tissues.

Retrospection

The role of temperature gradients in biological water transport in submerged plants was mentioned by RIEDE as early as in 1921. He thought to be possible that an outward water flow was maintained by the temperature of the plant tissues higher than that of their surroundings. He published data according to which the temperature of some hair-weeds was higher by 0.2-0.7 ^{o}C than that of the water, due to the oxidative processes within the plant's tissue. Unfortunately these ideas passed almost unnoticed, although recent experiments confirmed RIEDE's data. ERNST pointed out in 1936 (ERNST 1938; ERNST 1939) that there was a water flow across lipidic membranes from solution of higher to another one of lower temperatures even against a pressure. He accentuated the possible role of this phenomenon in biological systems. LIFSON and VISSCHER published a paper in 1951 in which they have written that thermo-osmosis was found in plant tissues. ERNST and HOMOLA performed various model experiments in 1952 and they suggested to explain the mobilization of hypo-osmotic liquids in biological systems by thermo-osmosis, they gave a number of examples for this. SPANNER (1954) thought that the heat of transfer Q^{*} that characterizes the thermo-osmotic effect was equal to the activation energy of osmosis, according to the basic principles of thermodynamics. Thus in his opinion a temperature difference of 1 ^{o}C can result in

a pressure of 132 bar (13.2 MPa) in the colder compartment of a plant tissue. NETTER (1959) assumed that thermo-osmosis played a significant role in biological systems. We reported at a national conference[2] and then in a paper (VETŐ 1963) that a small temperature gradient maintained experimentally in potatoes and apples drove water into the colder part of the plant tissue. BEAMENT (1964) and later O'DONNEL (1977) believed that temperature gradient played an important role in the process of water intake from the air in the case of certain insects. KATCHALSKY and CURRAN (1965) had the opinion: "... local temperature gradients cannot be completely ruled out and these might play some role in the transport of materials across biological membranes". DAVIES (1965) found this effect trivial, while ERNST (1966) argued against this opinion. VECLI and BIANCHI (1966) thought they found thermo-osmosis in frog skin in vitro. There is an osmotic pressure difference between the yolk and the white of an egg and very likely it is counteracted by thermo-osmosis in vivo, as it was found in our experiments (VETŐ 1966). We have measured the concentration of the bleeding sap produced by the root pressure in seedlings and the activation energy of the transport. Our results suggested that thermo-osmotic pressure might be considered the actual drive instead of the "active pressure of the plasm" (VETŐ 1967, 1981). Meanwhile quantitative model experiments proved that unlike it was thought by ERNST (1975) the difference of the vapour pressures was not enough as a driving force to explain the water transport (VETŐ 1967, 1969, 1974, 1982). HOUSE (1974) had a questionable scepticism: "There is no satisfactory evidence for or against thermo-osmosis in cells or tissues because practically no attention has been paid to this mechanism." The water transport caused by cryobiological processes is a determinant factor in the chance of the survival of cells. MEARES (1977) suggested the possibility of a correlation between heat and water flows in the discussion of FARRANT's paper (1977). The situation is the same in case of a foetus warmer then the mother's body (CONRAD and FABER 1977). HRYB (1981) believed that thermal energy was able to do work via thermo-osmosis in the respiration of mitochondria and peroxisomes. It is a possibility in case of water transport within trees (SKAAR and SIAU 1981; COHEN and OMI 1991).

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[2]Abstract: F. VETŐ, Acta Physiol. Hung. Suppl. 16, 50, 1959.

Experiments

1. Hen's egg

It is an old experience that it is easy to remove the shell of a hard boiled egg if it was put in cold water while it was hot.[3] It is obvious that a layer of liquid that occurs under the shell makes it possible. The question is that the temperature gradient between the flesh of the hard boiled egg and its surroundings can drive the water outwards throught the quasi-membrane formed by the gelatinous protein of the egg's white as it is predicted by the thermo-osmosis.

Methods. The hard boiled, hot eggs were wiped dry quicly, they were put in thin preservatives and they were kept at 0 ^{0}C in a refrigerator for 20 minutes to cool them. Then the rubber sack was removed and the liquid film below the shell was sucked into a syringe. The collected liquid between the membrane testacea and the hard ovalbumin gel amounted to 20-30 μl. Its osmotic concentration was determined by a Hewlett--Packard Vapour Pressure Osmometer. The weights of eggs were measured before and after they had been boiled and when we took them out of the refrigerator. In each case some juice was pressed from a sample of the ovalbumin gel of the same egg and its osmotic concentration was determined. NaCl solutions of known concentrations were used to verify the measurements. The time course of the temperature change was determined. The central part of the eggs was cooled from 75-80 ^{0}C to 35-45 ^{0}C in 20 minutes. Student's t-test was used to compare the data and to check if the difference was significant.

Results

1.1. The average osmotic concentration of the fluid between the membrana testacea and the hard ovalbumine was (180 ± 2) mOsm ($\pm$ S.E.M.), n = 46.

1.2. The juice pressed from the ovalbumine of the same 46 eggs has an average osmotic concentration of (223 ± 4) mOsm.

1.3. The differences of the above-mentioned data were calculated for each egg and their mean value amounted to (43 ± 3) mOsm, significant at a level of $P < 0.01$ as witness by a paired t-test.

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[3]Abstract: F. VETŐ, Acta Biochim. Biophys. Hung. 6, 483, 1971.

1.4. There was a loss of weight of eggs during boiling. The average decrease was (-0.34 ± 0.06) g, i.e. 0.7% of the initial weight (n = 45).

1.5. There is an additional small loss of weight after boiling till the end of the cooling, its mean value was (-0.020 ± 0.001) g, n = 45.

Conclusion

The osmotic concentration of the fluid under the shell of a hard boiled egg was less by 20% than that of the juice pressed out of the ovalbumine gel. This latter amounted to (223 ± 4) mOsm, the same as the value published for stored raw eggs, e.g. HEATH (1977). In other words, we found in osmotic model experiments that hypo-osmotic fluid was transported from the warm to the cold part of a gel system.

2. Paramaectum

Perhaps the most marked representation of the problem of the hypo-osmotic fluid transport in living world is the case of the pulsating vacuoles of freshwater Protozoa, as it was mentioned by ERNST (1939).[4] These cells have a cytoplasm of an osmotic concentration of 30-100 mOsm, and they pump the excess water out of the cell to the surroundings that have an osmotic concentration of 7 mOsm or so, this way controlling their internal concentration.

Questions: 1. The frequency of pulsation, does it depend on the concentration of the outer medium, 2. How does the enhancement (e.g. by DNP) of the chemical thermogenesis influence the frequency? 3. What is the effect of an experimental temperature gradient on the osmoregulation? 4. Has the thermo-osmosis a possible role in osmoregulation according to experimental results found in our laboratory and elsewhere?

Methods. The frequency of pulsation of the anterior contractile vacuole depended on the concentration as it was determined under a phase contrast microscope. Paramecia were cultured in the usual way then kept in Ringer's solution of various concentration (0-240 mOsm) under a coverglass. The times of a few pulsations were measured with a stop-watch and the frequency was calculated. A greater density of paramecia was achieved by centrifugation or filtering (glassfilter G.3) of the cultures. The suspension

[4]Abstract: F. VETŐ, Acta Biochim. Biophys. Hung. 3, 463, 1968.

of protozoa was washed with the test solution before the experiment. The measurements were done in animals paralysed by a solution of $Ni(NH_4)_2(SO_4)_2$ of a concentration of 25 mg% in order to avoid the difficulty caused by their ceaseless motion. This hardly influences the osmotic concentration of bath solutions, but the pulsation of vacuoles is continued even if at a frequency smaller by 30% than the normal one. The effect of 2,4-dinitrophenol (DNP) in a concentration of 10^{-5} mol/l on the frequency of pulsation was investigated in paralysed animals. The protozoa were soaked in the test solution containing DNP for 30 minutes before the measurement. Controls were treated in the same way but in solutions free of DNP.

We tried to use focused laser light to create intracellular temperature differences. Preliminiary experiments were performed in the laboratory of the Zeiss factory, Jena, with a ruby laser ZFL 750. The objective lens of the microscope focused the laser beam to a spot of a diameter of 30 μm in the object plane. This spot size is quite large compared to the diameter of a vacuole of 5 μm and it covers the third of the surface area of the animal. The maximum energy transferred in a single pulse was 0.02 J. We have investigated the effect of these laser pulses on paramecia stained with the vital dye methylene blue of trace quantity or on non-stained protozoa. The quantity of the absorbed energy was controlled by the concentration of the dye in the animal.

Results. The frequency of pulsation decreases with increasing concentration of both solutions over a range of 0-240 mOsm (0-1.0 n Ringer's solution).

Each point is the mean of 20 data, the $\pm$ S.E.M. values are indicated. An exponential curve was fitted to the points, the coefficient of correlation was -0.95 and the regression analysis resulted in the equation

$$n = 11 \exp(-2.7\ c)$$

where n is the number of pulsations in a minute, c is the normal concentration. The greatest part of the change of frequency occurred over a range of concentration 0-100 mOsm, and a slight change of frequency was only found above this concentration. To sum it up, the frequency of pulsation decreases exponentially with increasing concentration of the bath solution, as it is shown in Fig. 1.

The frequency of pulsation increased by 20% significantly ($P < 0.01$) under the effect of DNP which decouples the oxidative phosphorilation, stimulates the mitochondrial respiration and chemical thermogenesis but in-
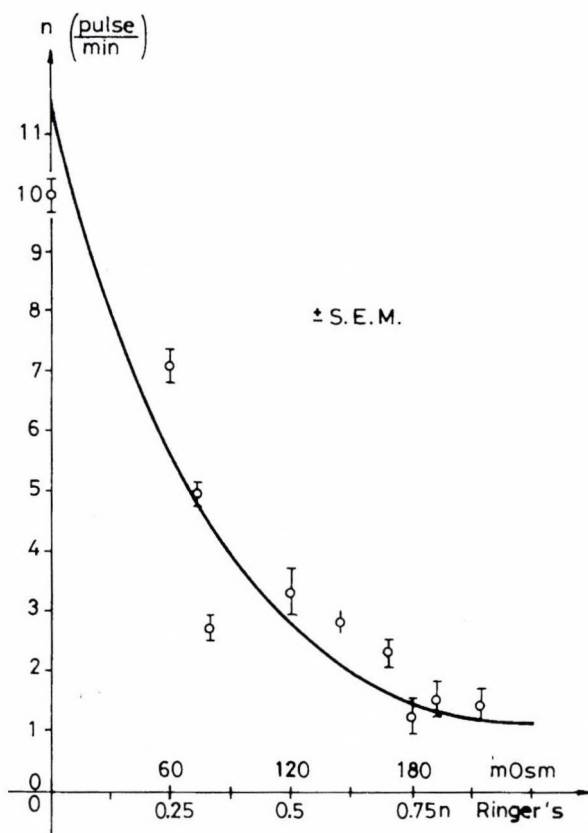
Fig. 1. Frequency of pulsation plotted against the concentration of the bath solution

hibits the synthesis of ATP. The mean frequency in control experiments was (3.9 ± 0.1) pulsations/min (the average of 206 data), which changed to (4.7 ± 0.2) pulsations/min (89 data) in the presence of 10^{-5} mol/l DNP.

It was impossible to achieve a selective increase of the temperature of the vacuole itself relative to that of the protoplasm, or inversely, because the diameter of the laser beam was greater than that of the vacuole. Our findings can be summed up, as follows. Unstained living paramecia were moving undisturbed and actively even after 5-10 laser shot, without the sign of any effect. Sometimes they were seen jumping when shot, the red light of the laser waser a stimulus for them. After 20-30 hits, their motion slowed, the protozoa swelled and disintegrated to small plasm balls. Methylene blue in trace quantities enhanced this effect. In some cases the part of the cell body hit by the laser shot exploded while the other part of the animal

survived and carried on moving for a few minutes. Strongly stained animals simply exploded at once after the laser shot. However, the effect of an intracellular temperature gradient has not been observed for technical reasons. Perhaps a better focussed beam of a CW laser could provide the required conditions unlike the pulsed laser did it.

Conclusions. Our experiments confirmed that the main function of vacuoles is the osmotic regulation, i.e. to remove the excess hypo-osmotic fluid, as it has already been verified many time. The enhancement of the chemical thermogenesis (mitochondria as microscopic heaters?) increased the frequency of pulsation. Therefore we can say the role of thermo-osmosis in the filling of the radial canals and ampoules is not unthinkable considering all the available data — the reversible contraction of volume caused by the absorption of laser light (SAKS et al. 1965), the great energy of activation (that is the great heat of transfer, Q^{*}) as calculated from the dependence of frequency on temperature (SPANNER 1954; KITCHING 1956; TSUKUDA and TAKEUCHI 1984). To accept this, we have to assume the existence of a localised, initial, intensive heat absorber. However, there is no direct proof of this, therefore the possibility of the role of thermo-osmosis in the case of pulsating vacuoles remains questionable.

3. Red blood cells

The red blood cells may be considered as osmometers in a rough approximation and they proved good experimental subjects to investigate the thermo-osmosis in living specimens.[5] The question is that their diameter (volume) or water content will change, i.e. thermo-osmosis occurs if the temperature of red blood cells is increased relative to their surroundings. We suppose that the temperature of red blood cells increases when their haemoglobin content absorbs energy of some incident light while the rise of the temperature of the medium is smaller because of the smaller rate of the absorption of the light energy. Another techniques of the selective heating of the erythrocytes is the treatment of the blood with ultrasound. The difference of temperature between the red blood cell and the medium can result in thermo-osmosis as it can be detected by the change of the diameter (volume) of the red blood cells.

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[5]Abstract: F. VETŐ, Acta Biochim. Biophys. Hung. Suppl. 2, 68, 1967.

Methods. We had no direct techniques (e.g. micro-thermocouple) to measure the temperature difference of the red blood cell and the medium, therefore we performed separate macroscopic experiments to demonstrate that the rise of temperature of the red blood cells is greater then that of the medium. Erythrocytes sedimented in a glass test tube were irradiated by intensive light. The temperature of the red blood cells was higher by 4 ^{0}C than that of the blood plasma. The packed cell volume of the red blood cells was determined in a micro-haematocrit centrifuge after they had been irradiated by intensive light. A significant decrease (5-10%) of the volume was found. Because a partial haemolysis might distort this result, we choose another method. The measurement of the diameter of single red blood cells proved to be a much more reliable method. The diameter decreased if the concentration increased, it was 90 scale marks of the eyepiece micrometer in NaCl solution of 0.6% and only 60 scale marks in NaCl solution of 1.8%. A difference was accepted as a significant one at a probability level of $P < 0.05$.

3.1. The diameter of red blood cells kept in solutions of various concentrations and illuminated by strong visible light from below (80 000 lux, through a water filter of 16 cm) was measured in a microscope with an eyepiece micrometer (100 scale marks = 7.3 μm). We could observe that some cells lost their haemoglobin content, they almost disappear with a ghost left behind. However, the great majority of them remained intact, these were measured.[6]

3.2. The effect of illumination on the volume of red blood cells was measured with Ljunberg Celloscope Counter, because there is a possibility — in principle — that while the diameter of the erythrocytes decreases, the bi-concave shape of red blood cells changes to a spherical one and the volume increases. Therefore, a more informative method is to measure the volumes in a direct way and to determine their distribution curve (Price—Jones). Blood was taken from the vein of ear of guinea pig and it was diluted 1:80 000 with a solution of NaCl of a concentration of 0.9%, then it was illuminated by a white light of 10 000 lux for 5 minutes before the measurement and during the measurement, as well, while infrared components were removed by a filter of a solution of $CuSO_4$. The same dilution of blood

[6]Similar preliminary experiments were performed on the naturally stained cells of the berry of privet (Ligustrum vulgare). The plasma sac never shrank but swelled under the effect of illumination's photolysis, decay of the plasma sac and disappearance of anthocyans occurred.

kept in darkness served as a control. The control suspension was kept in a water bath of a temperature higher by 2 or 3 ^{0}C than the ambient temperature, because the test suspension was warmed up to the same extent by the illumination.

3.3. A quartz piezo-crystal was the generator of the ultrasound ($f = 10^6$ Hz, $T_{max} = 6.10^4$ W/m^2) that was used to achieve a selective warming effect. The blood suspension to be treated in a thin rubber sac was hanged in the water above the vibrating crystal. Human blood treated with the anti-coagulant citrate and diluted 1:10 by a solution of NaCl of a concentration of 0.9% was used in this experiment. Duration and intensity of the ultrasound treatment were varied systematically. The diameter of red blood cells was measured before and afeer the insonation with a calibrated eyepiece micrometer in a microscope. The diameter of the cells was measured again in 30 minutes after the treatment had been finished in order to find out if the changes were reversible. The degree of an eventual haemolysis was determined by a spectrophotometer. The temperature of the blood suspension was raised to 36 ^{0}C during the ultrasound treatment.

Results

1.a. Diameter of red blood cells from heparinized dog's blood kept in hypo-osmotic NaCl solution of a concentration of 0.7%, various cells, measured in groups of 10 sample each

-- before illumination, a total of 378 measurement: (77 $\pm$ 1) scale units,
-- after illumination of 4 min, a total of 238 measurement: (74 $\pm$ 1) scale units.

The diameter decreased significantly by 4% (Fig. 2).

1.b. The diameter of human red blood cells from heparinized blood, suspended in a solution of NaCl of a concentration of 0.6% was (101 $\pm$ 1) scale units, average of 103 measurement; after an illumination of 6 minutes the average diameter decreased to (98 $\pm$ 1) scale units (103 measurements) and then, after keeping the samples in darkness for 5 minutes the average diameter became (102 $\pm$ 1) scale units (98 measurements). Therefore we can say that a reversible decrease of the diameter was caused by the illumination.

1.c. In a series of experiments 5 cells were identified in each view-field and the change of diameter was determined. Red blood cells of heparinized human blood were suspended in a NaCl solution of a concentration of
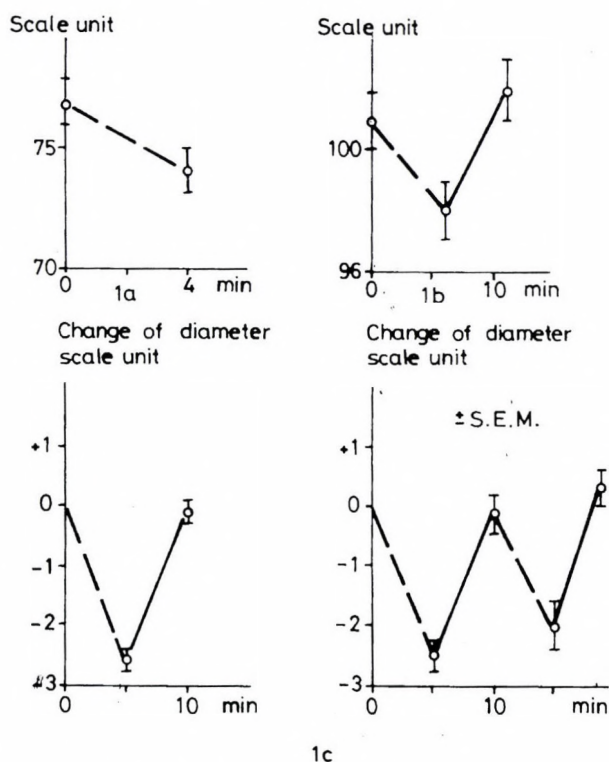
Fig. 2. Change of diameter of red blood cells illuminated (- - -) kept in darkness (——)

0.6%, diameters were measured before and after an illumination of 5 minutes; the average change of the diameters of 305 red blood cells was (-2.6 ± 0.2) scale units; the average change of the diameters of the same cells after keeping them in darkness for 5 minutes subsequently was (+2.5 ± 0.2) scale units. In another serial experiment both significant changes and reversibility were testified. The average changes of diameters were:

— after 5 minutes of illumination (-2.5 ± 0.3) scale units (n = 179),
— after 5 minutes in darkness (+2.4 ± 0.3) scale units (n = 178),
— after 5 minutes of illumination (-1.9 ± 0.4) scale units (n = 170),
— after 5 minutes in darkness (+2.3 ± 0.3) scale units (n = 159).

1.d. Citrated human blood was diluted by a 0.8 times normal mammalian Ringer's solution. It was illuminated for 5 minutes then kept in darkness for another 5 minutes. Controls were kept in darkness first then illuminated.
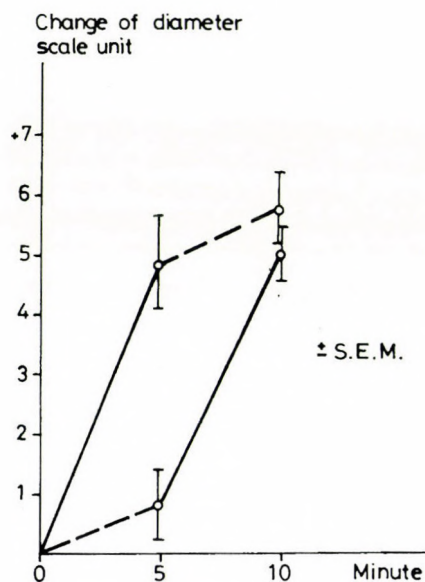
Fig. 3. Change of diameter of red blood cells illuminated (- - -) and kept in darkness (——)
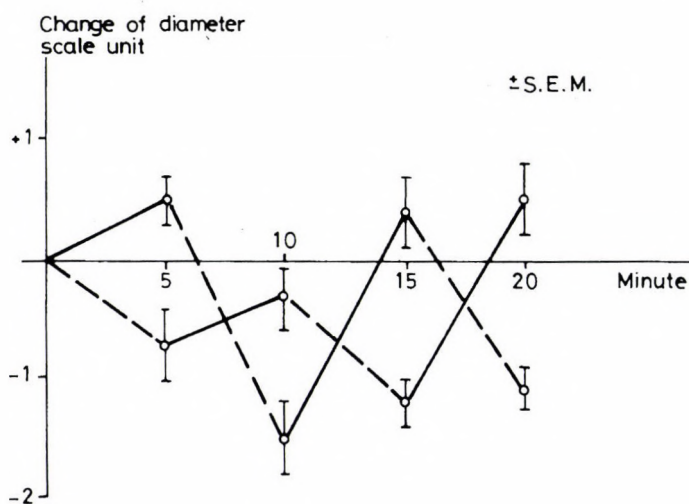
Fig. 4. Change of diameter of red blood cells illuminated (- - -) and kept in darkness (——) successively

The average change of diameter controls:

— 5 minutes in darkness (+4.9 ± 0.8) scale units (n = 45),

— 5 minutes, illuminated (+0.9 ± 0.6) scale units (n = 45).

The average change of diameter experiment:

— 5 minutes, illuminated (+0.8 ± 0.6) scale units (n = 48),

— 5 minutes in darkness (+4.3 ± 0.5) scale units (n = 48).

In this case, the rate of the increase of the diameter was reduced by intensive illumination (Fig. 3).

1.e. Citrated human blood was diluted by a NaCl solution of a concentration of 0.6% and it was illuminated by an intensive light for 5 minutes, then kept in darkness for another 5 minutes repeatedly. The changes of diameters of 5 identified cells were measured in each series of experiments (Fig. 4).

The average of changes were controls (230 cells):

— 5 minutes in darkness (+0.5 ± 0.2) scale units,

— 5 minutes illuminated (-2.0 ± 0.3) scale units,

— 5 minutes in darkness (+1.9 ± 0.3) scale units,

— 5 minutes illuminated (-1.5 ± 0.2) scale units.

The average of changes were experiment (235 cells):

— 5 minutes illuminated (-0.7 ± 0.3) scale units,

— 5 minutes in darkness (+0.4 ± 0.3) scale units,

— 5 minutes illuminated (-0.9 ± 0.2) scale units,

— 5 minutes in darkness (+1.7 ± 0.3) scale units.

As it can be seen, the cell diameter was decreased in a reversible way under the effect of repeated illumination by intensive light.

2. The discriminator unit D measured with a Celloscope counter is proportional to the volume of the cells under investigation, therefore it can represent the cell colume which significantly decreased by 6% under the effect of intensive light (50 D = 85 μm^3; 60 D = 102.4 μm^3). The data of 12 series of experiments (both illuminated and controls) are summarized in Table 1. The averages of volumes of illuminated and control cells were significantly different as tested by variance analysis and Fisher test. When we considered the original, direct counts (n) summarized them by units "D", the difference proved to be significant, too:

The average of controls, in darkness: (57.6 ± 0.4) unit "D"; n = 3641.

The average of illuminated cells: (54.1 ± 0.4) unit "D"; n = 3244.

The summarized distributions of the volumes are shown in Fig. 5, as the per cent of the total of counts for controls and illuminated cells.

Table 1

Effect of illumination on the volume of red blood cells, measured with Celloscope

Serial number	Controls in darkness			Illuminated		
	Total of counts (n)	Volume D_d	± S.E.M.	Total of counts (n)	Volume D_l	± S.E.M.
1.	485	63.1	0.9	261	49.7	1.2
2.	579	49.4	0.8	387	40.1	1.0
3.	325	48.1	1.1	342	48.2	1.0
4.	414	53.1	1.0	326	51.2	1.1
5.	358	42.6	1.0	206	42.2	1.3
6.	62	46.8	2.5	160	49.8	1.5
7.	142	43.4	1.6	258	37.0	1.2
8.	200	41.2	1.4	200	54.6	1.4
9.	246	72.6	1.2	273	68.5	1.2
10.	311	74.1	1.1	252	73.6	1.2
11.	216	71.1	1.3	217	66.0	1.3
12.	303	78.7	1.1	362	69.4	1.0

$\bar{D}_d = 57.6$ $\bar{D}_l = 54.1$

$$\frac{(\bar{D}_l - \bar{D}_d) \cdot 100}{\bar{D}_d} = -6\%$$

$F = 124$ $P \ll 0.001$

$F_p = 2.44$ $P < 0.001$

3. The changes of the diameter of red blood cells produced by the selective heating effect of the ultrasound are summarized in Table 2. It was found that the diameter of non-haemolized red blood cells was reduced by ultrasound treatment, and this change was reversible. The time duration of ultrasound treatment and the relative decrease of the diameter were rather closely correlated, the coefficient of correlation was +0.91 ± 0.08; i.e. a longer treatment resulted in a bigger decrease of the diameter.

No decrease of the diameter was found when the cell suspension was heated in a warm-water bath without ultrasound treatment. Therefore the effect found in the experiments reported here was very likely caused by the selective warming action of the ultrasound treatment. The average diameter of 550 red blood cells was (37.6 ± 0.2) scale unit at room temperature and (37.3 ± 0.2) scale unit was the average diameter of 540 red blood cells at a temperature of 36 ^{o}C ($0.20 < P < 0.50$).

Conclusion. The ultrasound had an effect which was practically the same as that of the light represents an energy flow of another character. Both forms of energy transfer resulted in a reversible decrease of volume of red blood cells, because these radiation of different microphysical char-
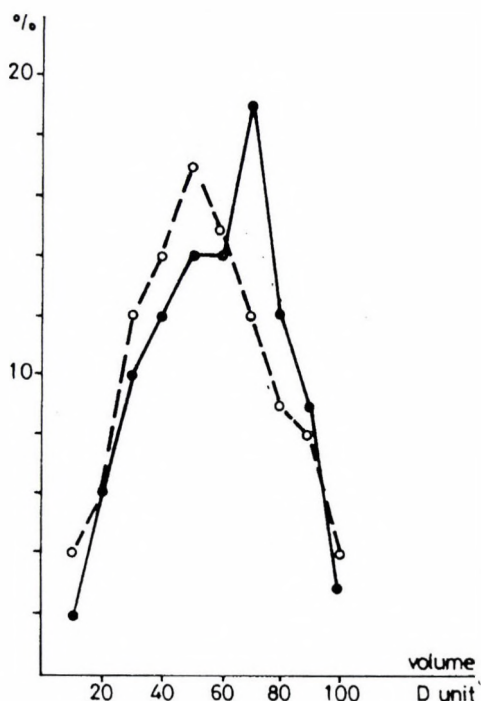
Fig. 5. Distribution of volumes of red blood cells illuminated (- - -) and kept in darkness (——)

acter have a similar selective warming action. The reversibility suggests that a thermo-osmotic change of volume occurs in these experiments, although we have not got data which could prove or disprove that some transfer of dissolved substances joins to the water-transport associated with the change of volume. The percentually small change of diameter is a significant effect because, e.g. a decrease of 4% of the diameter of disc corresponds to a decrease of 8% of the volume ($0.96^2 = 0.92$) even if the thickness of the disc does not change, and, in turn, it corresponds to 16% of the water that can be mobilized osmotically. All these result in a significant increase of concentration. The energy absorbed by a single cell is not known. It would be exaggerated to give further estimates of the magnitude of temperature differences in the investigated system and of the heat of transfer (Q^*). Further investigation and more sophisticated techniques would be required to answer these questions. However, our results are good enough to support the assumption of the existence of thermo-osmosis in these cases.

Table 2

Effect of ultrasound on the diameter of red blood cells

Power	4 W/cm²				6 W/cm²
Time of treatment	1/2 min	1 min	3 min	6 min	3 min
Number of cells investigated in groups 10 cells each	120	90	140	150	290
Initial diameters scale units ± S.E.M.	38.3 ± 0.3	36.2 ± 0.2	38.3 ± 0.4	40.4 ± 0.4	43.9 ± 0.4
Diameters after treatment	37.8 ± 0.3	35.4 ± 0.3	37.0 ± 0.4	38.9 ± 0.4	40.1 ± 0.3
Diameters in 30 min	38.9 ± 0.4	36.6 ± 0.4	39.1 ± 0.4	40.6 ± 0.4	42.6 ± 0.5
Degree of haemolysis of controls %	3	3	5	5	5
Degree of haemolysis of treated cells %	18	17	17	50	23
Percentual change of diameter just after treatment:	-1.3	-2.2	-3.4	-3.7	-8.7
in 30 min:	+2.9	+3.3	+5.5	+4.2	+5.7
Temperature °C after treatment	27	28	30	32	33

Discussion

We have found that

— a transport of hypo-osmotic liquid occurred from the warm to the cold in the gel system of hen's egg,

— the enhancement of the chemical thermogenesis increased the pulsing frequency of the vacuoles responsible for the control of the osmotic system of paramecia,

— irradiation by intensive visible light or ultrasound resulted in a reversible decrease of the volume of red blood cells.

All these suggest that experimental thermo-osmosis may be brought about in living systems in vitro and we cannot exclude its existence in vivo either, however, further investigations are required to find out the details of this problem. However, we feel that our investigations gave remarkable points to the understanding of the water metabolism of protozoa (TSUKUDA and TAKEUCHI 1984; COUILLARD 1986; IKEDA and TSUKUDA 1989) and of red blood cells (MACEY 1984; NICCOLAI et al. 1984; LING and OCHSENFELD 1986; BRAHM and

GALEY 1987; SULLIVAN et al. 1988; OJCIUS and SOLOMON 1988; BENGA 1988; YE and VERKMAN 1989), as well as to their reactions to illumination (POOLER 1985) by visible light. We do not deal with the exciting question of the state of water in cells, but we want to refer to the possibility that the effect of the ultrasound is manifested via modification of the dynamic structure of water (SEHGAL and GREENLEAF 1986; SÜSS 1988). Another exciting problem to be solved is to clarify what is the connection between the increase of the biochemical endogen thermogenesis and the water exchange (control of volume), e.g. in mitochondria, in case of brown adipose tissue (AMIR and DE BLASIO 1991), in birds' red blood cells having nuclei or in red blood cells with arsenate. A newly developed thermal microscope of scanning probe could give way to the thermal mapping of living cells.

Our final conclusion is that thermo-osmosis can be produced in living systems in vitro.

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The author commemorates the late professor Eugene Ernst who was his master in science and who initiated his work in this field. The careful laboratory work was done by Gabriella Bod and later by Anna Geges Mrs Horváth and Gabriella Török Mrs Metzger. Marianna Petro dr, as an undergraduate was an enthusiastic participant of this work.

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Acta Botanica Hungarica 38 (1—4), pp. 221—226 (1993—94)

OCCURRENCE, GENESIS AND ULTRASTRUCTURE OF ANTHOCYANOPLAST-LIKE STRUCTURES IN CELLS OF CALLUS CULTURE OF DROSERA SPATHULATA LABILL.

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Spherical dense bodies — anthocyanoplasts from pigmented callus cells of Drosera spathulata were observed. Some of them took place in the cytosol, others in the vacuole. More detailed analysis has shown that they represented intracellular compartment. The synthesis and accumulation of anthocyanin and formation of bodies from dense substances as well were connected with active functioned endoplasmic reticulum (ER). Final translocation and deposition of electron dense bodies had been associated with tonoplast and vacuoles, where we were able to see freely floating osmophilic aggregates.

Introduction

Considerable attention is focusing to examine different types of organelles in intact plant cell and cell cultures up to date. But the contributions, concerned of genesis and ultrastructure of anthocyanoplasts, which probably represent sites of anthocyanin biosynthesis and accumulation are relatively scarce. Intensively pigmented structures (anthocyanoplasts) have been reported in young anthocyanin-containing cells of more than 70 species (PICKET and SMALL 1980; SMALL and PICKET 1982). Existence of these structures in cell suspension cultures of sweet potato were described by NOZUE and YASUDA (1985) and in cells of willow tissue culture by LIŠKOVÁ et al. (1989). The ultrastructure and occurrence of anthocyanoplasts of callus culture of Drosera spathulata together with their genesis during the process of callogenesis were studied in the present work.

Material and Methods

Cultures were established from leaves cultivated on basal MS medium (MURASHIGE and SKOOG 1962) and supplemented by growth substances (0.01 $mg \cdot l^{-1}$ or 1 $mg \cdot l^{-1}$ 2,4-D and 1 $mg \cdot l^{-1}$ kinetin) as well as 5% coconut milk. Isolated leaves were cultivated in Petri dishes in

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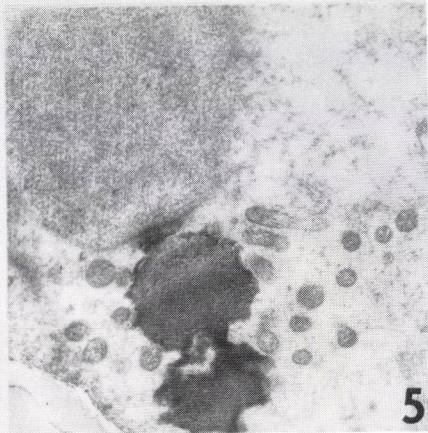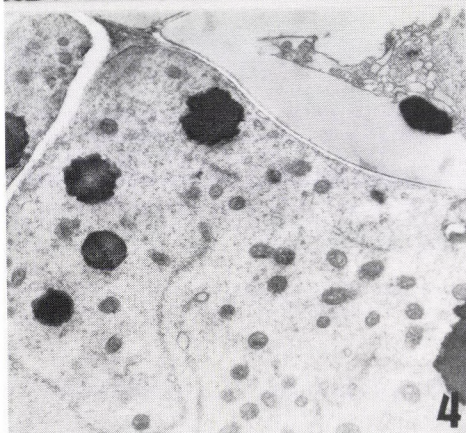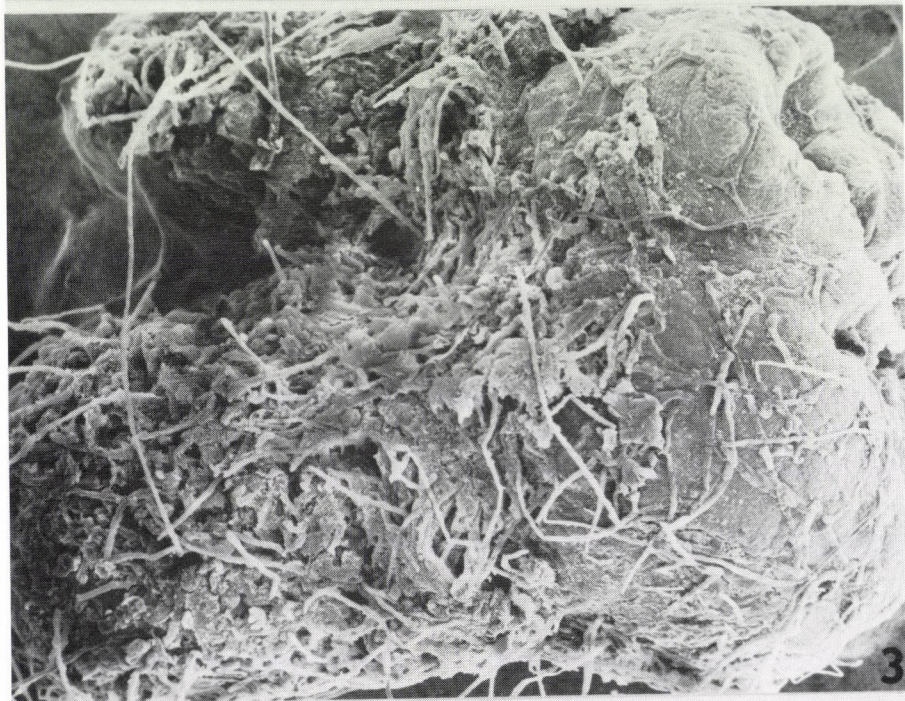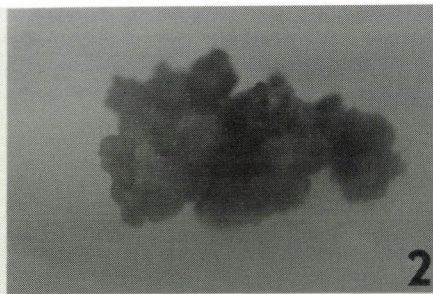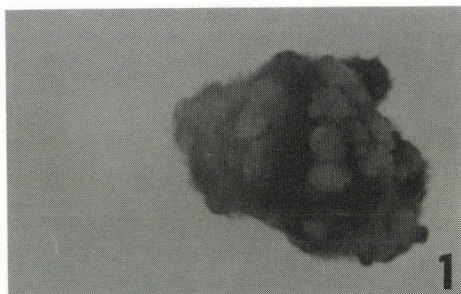
cultivating chamber. Part of the material was cultivated in long day conditions (16 hours light) and the other one in dark at the temperature 24 ± 1 °C. The calli were passaged monthly to fresh medium lacking auxin. The calli cultivated in the light in long day conditions were dark green and very hard (Fig. 1). Material for cytological and ultrastructural studies had been taken from pigmented parts of the calli after 20 days of subcultivation in dark (Fig. 2). Some areals of the colour calli were covered by root hair-like structures (Fig. 3). The material was fixed by 5% glutaraldehyde in a phosphate buffer and 2% OsO_4 solution for 2 hours. Samples were embedded into Durcupan ACM. Ultrathin sections were contrasted by lead citrate according to VENABLE and COGGESHALL (1965). There after they were studied in the electron microscope JEOL 2000 FX.

Results and Discussion

Plant tissue and cell cultures have several advantages in comparison to whole plants and organs, because they are able to synthetize specific compounds, especially various secondary metabolites. Some of them are used as medicine, food, colouring reagents etc. (TANAKA 1989).

Using of plant tissue cultures for production of secondary compounds have been a major aim of our recent research (BOBÁK et al. 1989; BLEHOVÁ et al. in press). The carnivorous plants of Drosera species are a good source for production of important substances as 1,4-naphtoquinones especially plumbagin and 7-methyljuglone, which are used recently as parts of drugs with antisclerotic effects in the pharmaceutical industry. On the other hand they are able to synthetise some pigments as anthocyanin and other flavonoides as well. Relatively few information is available about the site of anthocyanin biosynthesis and genesis of anthocyanoplasts, which are probably sites of the later stages of anthocyanin biosynthesis and accumulation. There is evidence that anthocyanin is synthetized and packed near the endoplasmic reticulum and then transported into vacuole (HRAZDINA et al. 1978). Some authors prefer an alternative view that the anthocyanin is synthetized in plastids (RAUJEVE et al. 1977). But others reported about the cytosol or vacuole as a probably site of biosynthesis of anthocyanin. Most of the

Fig. 1. Drosera tissue culture after 20 days of cultivation in the light

Fig. 2. Drosera tissue culture after 20 days of cultivation in darkness

Fig. 3. SEM shiwing detail of root-hair formation like structure on the surface of calli. Magnification: 2500x

Fig. 4. Genesis of small vesicles from endoplasmic reticulum. Magnification: 7000x

Fig. 5. Single stages of differentiation of anthocyanoplast-like structures. Magnification: 15 000x
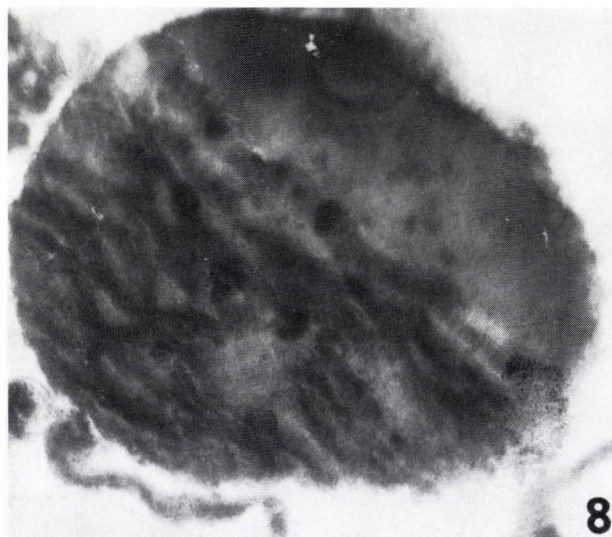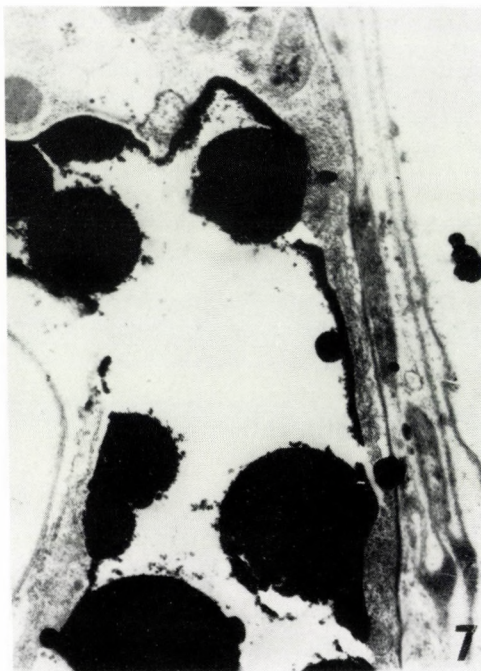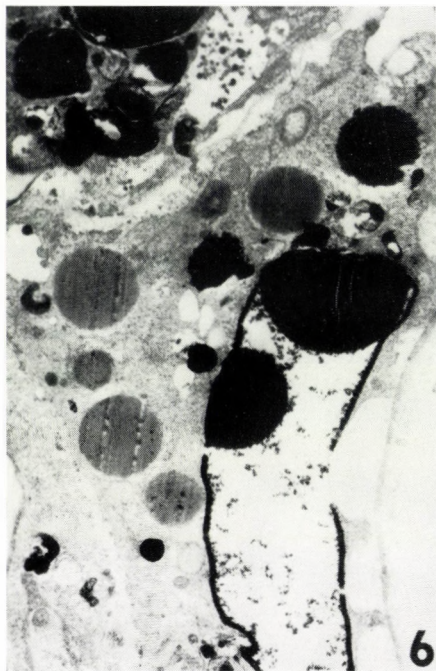
Fig. 6. Occurrence of large anthocyanoplast-like structures in cytosol and vacuoles. Magnification: 14 000x

Fig. 7. Aggregation of large bodies in vacuoles. Magnification: 17 000x

Fig. 8. Section through the anthocyanoplast-like structure — detail viewing. Magnification: 32 000x

authors suggest that some enzymes involved in anthocyanin biosynthesis occur in above-mentioned compartments.

Cytological investigation of calli which grew on different cultivation media revealed existence of red pigmented cells with different colour intensities. Visual appearance of pigmented cells were noted within 4 weeks after transfer of calli on the cultivating media. Electronmicroscopical analysis showed that existence of these cells were connected with presence of intensively pigmented numerous anthocyanoplasts (dense bodies), which were found in these cells. Some of them occurred in the cytosol, others were found in the vacuole. Our recent interest is focused on anthocyanoplast biogenesis.

The synthesis, accumulation and formation of bodies from dense substances is connected with active function of endoplasmic reticulum (ER). Dense substances are noticeable in vesicles or sacs associated with endoplasmic reticulum. It is very common to find cisternae of endoplasmic reticulum very close to formed bodies (Fig. 4). On the electronogram (Fig. 5) is a group of small vesicles piching from endoplasmic reticulum cisternae dense substances. There are gradually transformed into large bodies — anthocyanoplasts. Some of them are located in cytosol (Fig. 6), but part of dense substances are inserted into vacuolar system and formed clusters associated with tonoplast. The bodies are formed later by the fusion of this dense material. Dense substances are accumulated before in some protuberances in cytosol. Localization of dense substances and dispersion of precipitate were observed in the irregular shaped vacuole (Fig. 7).

Very often freely floating bodies can be find within the vacuolar space. This is in agreement with reported data of HRAZDINA et al. (1978). The number of the bodies varies from one to ten per one cell. Relatively often a group of anthocyanoplasts of different size and shape was found. They occupy a considerable part of the cell volume. More detail electronmicroscopic analysis of the anthocyanoplasts have shown that their stroma, which sometimes content the osmophilic globules (Fig. 8) is of higher density than the cytosol. Fully developed anthocyanoplasts reached 10-20 μm in diameter after 30 days of cultivation of callus culture.

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ISOLATION AND CHARACTERIZATION OF BACILLUS STEAROTHERMOPHILUS STRAINS FROM A THERMAL WELL OF THE UPPER-TRIAS WARMWATER SYSTEM IN THE REGION OF BUDAPEST (HUNGARY)

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Thirty-six representative strains were selected for detailed studies from among our Bacillus isolates obtained from the water (76 °C) of the "Szent István" well (thermal well No. II of the Széchenyi bath, Budapest). According to our results the water of this well contained a homogeneous population of a local variety of Bacillus stearothermophilus. This is the first true thermophilic bacterial species which has been isolated from the extensive thermal water systems in the region of Budapest.

Introduction

The research work on the distribution, activities and taxonomy of microorganisms colonizing the subsurface and spring waters is at present in the foreground of many laboratories. Especially thermophilic bacteria and other microorganisms adapted to extreme environmental conditions are the subjects of thorough investigations. Thermophilic microbes are mostly isolated from the waters of hot springs (BROCK 1978; KRISTJANSSON et al. 1986; SISSONS 1987; HUDSON 1989; BONCH-OSMOLOVSKAYA 1990, etc.) because the smapling of the deeply migrating ground waters frequently encounters insoluble difficulties. Among the moderate thermophiles (temperature optimum of growth of these microbes is between 55 °C and 75 °C) were mostly bacilli and actinomycetes presented as typical colonizers of certain types of thermal springs. The most intensively studied species of such thermophilic bacilli is at present Bacillus stearothermophilus (YANAGITA 1990).

Thermophilic bacteria frequently occur in the water of curative thermal bath, hot springs the water of which is used for drinking and curative thermal muds employed for therapeutical purposes. These bacteria could produce e.g. water soluble metabolic products, which could positively or negatively influence the quality of the thermal water. The region of Budapest is rich in thermal spas, springs and wells and their water attract in

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every year many thousands of ill and healthy people. Unfortunately, at present only very few is known on the indigenous bacterial communities of these thermal waters. The aim of our effort was to carry out a series of microbiological analyses on the thermal wells' waters of the Hungarian Capital and its surroundings. Below we present the results of our first studies.

Material and Methods

The studied thermal well was well No. II of the Széchenyi bath in Budapest, protected by a small well-building in which the pipe of the water was at times closed or opened. The water rich in calcium and magnesium salts has been elevated to the surface, through a pipe system, from the calcareous and dolomitic aquifer (ground deepness of the well bored between 1936 and 1938 is 1246 m) sedimented and developed during the Upper-Trias period.

Chemical characteristics of the water. The water of the well has been characterized by the laboratory of the Baths' Direction as a typical calcium-magnesium-hydrogencarbonate one. In this mineral water predominate, according to their equivalent per cent, in contrast to the alkalies (Na^+ and K^+; 8.96 mg equiv. expressed in Na^+) the earthalkali metals (Ca^{2+} 7.61 mg equiv., Mg^{2+} 3.15 mg equiv.). Furthermore the equiv. per cent of hydrogencarbonates proved to be higher (HCO_3^- 9.21 mg equiv.) than that of the sulphate and chloride (SO_4^{2-} 4.45 mg equiv., Cl^- 5.91 mg equiv.). The presence in the water of this well of calcium-, magnesium- and hydrogencarbonate-ions is characteristic for the so-called karst-waters.

Sampling of the well's water. Before collecting the water from the pipe-system of the well for bacteriological analyses the surfaces of all metallic constituents of the tap were sterilized by flame and the water was let flow away during at least five minutes. Water samples were aseptically collected in sterile Erlenmeyer-flasks and transported without changes in their temperature into laboratory where they have been elaborated still at the sampling day.

Isolation of bacteria. Four different methods were employed for the isolation of the thermal-water bacteria. All of these methods has been associated with the use of the same four isolation-media: a) from the well-water prepared simple "water-agar", b) "water-agar" supplemented with 0.2% glucose and 0.017% $NaNO_3$, c) yeast-extract starch agar, and d) meat-extract peptone agar.

Method No. 1. Undiluted well-water samples were distributed in 0.1 ml volumens on the surfaces of simple water-agar plates in Petri dishes and the latters were incubated for isolations.

Method No. 2. 0.5—1.0 l volumens of the well-water were filtered through membrane-filters of 0.45 μm pore diameter. The outfiltered bacterial cells and spores bearing membrane filters were placed on the surfaces of agar plates of the four isolation media in Petri dishes and incubated.

Method No. 3. For estimating the germ number of microaerophilic- and anaerobic bacteria, melted and to 50 °C cooled agar-media in Burri tubes were also inoculated with untreated thermal water and incubated.

Method No. 4. The original and generally low germ number of bacteria in the thermal water has been increased before plating by mixing (1:1) and incubating the water samples with nutrient broths of different chemical composition: a) thermal water broth containing 0.2% glucose and 0.017% $NaNO_3$, b) meat-extract peptone broth, and c) yeast-extract starch broth.

The inoculated agar-plates and the media in Burri tubes were incubated at 68 ^{o}C temperature. As the best medium for isolation purposes proved to be the yeast-extract starch agar. The appeared colonies were picked up and transferred onto slants. Pure cultures were obtained on yeast-extract starch agar (YESA) plates by reisolations. The maintenance of the strains were carried out by continuous cultivations and transfers in every month one times on YESA slants. The first period of cultivation lasted 24 hours at 65 ^{o}C and the second one at about 28-30 days at room temperature.

Studied differential-diagnostic properties. Gram staining with 24 hours old cultures, decolourization with 96% ethanol and differential staining with 1% safranine solution; Macromorphological studies on the colonies growing on different media; Light microscopic observations on the cell morphology using the Gram stained smears; Detection of the presence of endospores in the cells of a 5 days old culture the smears of which were heat treated with 5% malachitgreen and there after counterstained with 1% safranine; The ability of active movement was tested in YESA stab cultures; Catalase production was shown in 24 hours old YESA cultures with 10% H_2O_2 solution; Oxidase activity has been detected by treating 24 hours old cultures on YESA plates with 1% aqueous solution of p-aminodimethylalanine-monohydrochloride; The fermentative versus oxidative decomposition of glucose (O-F test) was tested using the method of HUGH and LEIFSON (1957). For this purpose employed basal medium: peptone 2.0 g, NaCL 5.0 g, K_2HPO_4 0.3 g, agar (Oxoid) 3.0 g, dist. water 1000 ml, 0.2% bromothymolblue solution 15 ml, pH 7.0, glucose was sterilized by Seitz EK-filtration, the anaerobic tubes were covered by paraffine, inoculation by stab, incubation lasted 3 days; Growth under anaerobic conditions in anaerostat on Difco Tryptic Soy Agar, check of the growth after 24 and 48 hours; Growth on Simmon's citrate agar; Utilization of propionic acid as sole source of carbon on modified Koser-agar: NaCl 1.0 g, $MgSO_4 \cdot 7\ H_2O$ 0.2 g, $(NH_4)_2HPO_4$ 0.5 g agar (Oxoid) 15.0 g, dist. water 1000 ml, 0.04% aqueous phenolred solution 20 ml, pH 6.8, propionate was added at the final conc. of 0.2%, the slantagar-cultures were incubated till 3 days; Caseinase activity was studied on with Bacto Skim Milk (2.5%) prepared nutrient agar plates. After 2 days incubation the inoculated plates were treated with acid $HgCl_2$ solution; Gelatinase activity has been detected on gelatin-agar plates: gelatin 4.0 g, meat-extract 3.0 g, peptone 5.0 g, agar(Oxoid) 15.0 g, dist. water 1000 ml, pH 7.0. After 3 days cultivations the plates were flooded with acid $HgCl_2$-solution; Growth in nutrient broth at pH 5.7 and 6.8 values; Tolerance to 3, 5, 7 and 9% NaCl in YES-broth; H_2S-production from peptone in Difco pepton Iron Agar (5 days incubation) and nutrient broth (NaCl 5.0 g, meat-extract 5.0 g, peptone 10.0 g, dist. water 1000 ml, pH 7.0, 3 days incubation), the H_2S was detected with sterile paper stripes impregnated with lead-acetate, and hang over the culture surfaces; Production of indole in peptone-water (NaCl 5.0 g, peptone 10.0 g, dist. water 1000 ml, pH 7.0), the Kovács-reagent was used to detect the presence of indole after 2 days incubation; From peptone-water the NH_3 was also shown with the aid of Nessler-reagent; In Difco MR-VP medium (peptone 7.0 g, glucose 5.0 g, K_2HPO_4 5.0 g, dist. water 1000 ml, pH 6.9) after two days incubation acetonin was detected with Barrit-reagent; Nitrate reduction: after 3 days incubation in nitrate containing nutrient broth (meat extract 3.0 g, peptone 10.0 g, KNO_3 1.0 g, dist. water 1000 ml, pH 7.2) nitrite was showed by Griess—Ilosvay-reagent and the presence of the remained nitrate was detected with the help of zinc dust; Phenylalanine deaminase activity was studied after 3 days incubation on the basal medium (DL-phenylalanine 1.0 g, yeast-extract 3.0 g, Na_2HPO_4 1.0 g, NaCl 5.0 g, agar(Oxoid) 15.0 g, dist. water 1000 ml, pH 7.2) with the help of a 10% $FeCl_3$ solution; For presenting the amylase production the strains were cultivated on starch-agar plates: soluble starch 10.0 g, peptone 5.0 g, meat-extract 5.0 g, NaCl 5.0 g, agar(Oxoid) 15.0 g, dist. water 1000 ml, pH 7.0. These were treated after incubation with Lugol solution; The temperature range of growth on yeast-extract agar (yeast-extract 5.0 g, peptone 5.0 g, glucose 10.0 g, agar(Oxoid) 15.0 g, dist. water 1000 ml, pH 7.0) was tested at 40 ^{o}C, 50 ^{o}C and 60 ^{o}C (incubation: 3 days) furthermore at 65 ^{o}C, 70 ^{o}C, 76 ^{o}C and 80 ^{o}C (incubation: 1 day) temperature values; Urease activity was tested on Christensen's agar slants (peptone 10.0 g, glucose 1.0 g, NaCl 5.0 g, KH_2PO_4 2.0 g, agar 15.0 g, 2% phenolred solution 6 ml, dist. water 1000 ml, pH 6.8). The through Seitz EK-filter sterilized 20% urea solution was added to the basal medium in a such volumen that the final urea concentration reached 2%; The ability of the strains to hydrolyse Tweens (-20, -40, -60 and -80) has been studied in peptone-agar (peptone 10.0 g, NaCl 5.0 g, $CaCl_2 \cdot 2\ H_2O$ 0.1 g,

agar(Oxoid) 15.0 g, dist. water 1000 ml, pH 7.0—7.4). Tweens were added in 1% concentrations. After two days incubation the appearance of opaque zones of fine chrystals around the colonies were observed; Methylene-blue reduction in tryptone broth (tryptone 5.0 g, meat-extract 3.0 g, 0.1% methylene-blue solution 6.6 ml, dist. water 1000 ml, pH 7.0). Incubation and continuous observations lasted 2 days; Utilization of carbon compounds (arabinose, ribose, xylose, rhamnose, fructose, galactose, glucose, mannose, lactose, sucrose, trechalose, raffinose, dextrin, dulcitol, glycerol, inositol, mannitol, sorbitol, ethanol and methanol) as sole sources of carbon on synthetic basal medium: NaCl 1.0 g, $MgSO_4$ 0.2 g, $(NH_4)_2HPO_4$ 1.0 g, KH_2PO_4 5.0 g, agar (Oxoid) 15.0 g, 0.04% phenolred solution 20 ml, dist. water 1000 ml, pH 6.8—7.0). The majority of tested compounds were sterilized by Seitz-EK filtration. Incubation lasted 3-5 days and then recording of the results on the basis of comparisons with the growth on the control without C-source; Sensibility against lysozyme was tested in nutrient broth (peptone 10.0 g, meat-extract 10.0 g, NaCl 5.0 g, dist. water 1000 ml, pH 7.0) in which through Seitz EK filter sterilized lysozyme was added at 0.01% concentration. Incubation lasted 24 hours and then a comparison was carried out with the growth in broths without lysozyme; Growth in Difco Azid Dextrose Broth was observed after 24 and 48 hours incubation; DNase and RNase activities were tested on casamino acid agar medium (glucose 5.0 g, casamino acid vitamin free 5.0 g, K_2HPO_4 5.0 g, NaCl 2.0 g, $FeSO_4$ 0.05 g, $MgSO_4$ 0.5 g, agar (Oxoid) 15.0 g, DNA or RNA 2.0 g, dist. water 1000 ml). After inoculation the plates were incubated 24 hours and treated with 1N HCl solution; Decomposition of tyrosine and hypoxanthine were studied in nutrient agar (meat-extract 3.0 g, peptone 5.0 g, agar (Oxoid) 15.0 g, dist. water 1000 ml). The separately heat sterilized tyrosine and hypoxanthine suspensions were added into the medium at a final concentration of 0.5%. Incubation lasted 48 hours; Nutrient agar (1000 ml) supplemented with through Seitz EK-filter sterilized 1% Na-phenolphtalein-diphosphate-solution (10 ml) was used to detect the phosphatase activity. The incoulated and incubated agar plates were exposed to the vapour of ammonia; Acid production from by filtration sterilized carbohydrates (arabinose, ribose, xylose, fructose, glucose, mannose, lactose, sucrose, dextrin, glycerol, inositol, mannitol, sorbitol, ethanol and methanol) was tested on yeast-extract agar: yeast extract 0.2 g, $(NH_4)_2HPO_4$ 1.0 g, KCl 0.2 g, $MgSO_4 \cdot 7\ H_2O$ 0.2 g, agar (Oxoid) 15.0 g, 0.04% bromocresolpurple 15 ml, dist. water 1000 ml, pH 7.0). C-source in the medium was given in 1%. A comparison was carried out with the negative control after 3 days incubation; Resistance against antibiotics and antibacterial substances: 24 hours old nutrient broth cultures were suspended and distributed onto nutrient agar plates and immediately after this inoculation paper discs impregnated with given amounts of antimicrobial substances were placed on them. Incubation lasted 24 hours and the radius of zones around the discs without growth of the test organism was recorded. The following antimicrobial compounds were tested: ampicillin 20 mcg, carbenicillin 50 mcg, chlortetracycline 30 mcg, chloramphenicol 30 mcg, clindamycin 10 mcg, erythromycin 10 mcg, gentamycin 20 mcg, kanamycin 30 mcg, lincomycin 10 mcg, nalidixic acid 30 mcg, neomycin 100 mcg, nitrofurantoin 300 mcg, nystatin 100 IU, oleandomycin 30 mcg, oxacillin 10 mcg, oxytetracycline 30 mcg, penicillin 3 IU, polymyxin-B 15 mcg, spiramycin 30 mcg, sumetrolim 25 mcg, superseptyl 400 mcg, tetracyclin 30 mcg, vancomycin 50 mcg.

Detailed descriptions of the above listed methods are published in the works of CLAUS—BERKELEY (1986), COWAN and STEEL (1974), SZABÓ (1974) and GORDON (1973).

Results and Discussion

From the point of view of the growth and activities of microbes in the thermal water the temperature and the O_2 content are key parameters. The water temperature of the well changed at the time of our sampling between 75.8 and 77.3 ^{o}C, while the presence of oxygen dissolved in the water was not detectable by using the method of Winkler. This latter statement is also in accordance with data in the literature (SCHULHOF 1957).

Using the plate count technique for germ number estimates the total number of bacteria in 0.1 ml of the undiluted original well water proved to be extremely low, practically not exactly calculable. Employing the membrane filter technique we could stated that after passing through the filter about 1-1.5 l well water only 4-10 viable bacterial colony forming units remained on the surface of a filter membrane. Furthermore this latter results were only obtained if we used nutrient media with organic C- and N-sources for cultivating the filtered germs. We estimated on the basis of a series of measures the average germ number content of this thermal water to about 10/l. In the media of the closed Burri tubes bacterial developments have never been observed. The majority of the isolates were obtained on aerobically cultivated YESA-plates, but some isolates were also picked up from meat-extract-peptone agar plates, which were inoculated with glucose and $NaNO_3$ supplemented and precultivated well water. From among our isolates we carried out detailed diagnostic studies on 36 representative strains. All of these latters were included, on the basis of their differential-diagnostic features, into a single homogeneous assemblage of very similar strains. On YESA slants or plates the cultures of these catalase positive strains frequently produced creamy coloured, easily smearable colonies of glistening surface. Soluble pigments were by them not excreted. Their Gram variable, 3-6 μm long, rod shaped cells developed oval endospores of 1 x 2 μm size. The actively moving vegetative cells could form especially in the logarithmic phase of their growth also more than 30 μm long chains. All of them proved to be the typical members of the genus Bacillus. The comparative data in Table 1 clearly demonstrate that our isolates belonged to Bacillus stearothermophilus, a species which is widely distributed in the nature, possesses a limited tolerance against acid environmental conditions, but its members are able to grow at 76 ^{0}C temperature. Bacillus stearothermophilus is, however, a very variable so-called "collective species" which comprises many ecotypes and local varieties, which perhaps later will be considered as representatives of separate, new species of Bacillus. This hetereogeneity is the "cause" of the existence of its many supposed phenotypes and the diversity in the DNA's base-composition of its members. The latter can partly be trace back to its already demonstrated abilities to base transfer (LEVY 1989).

Our thermal-well-B. stearothermophilus isolates can ferment glucose, produce acids form carbohydrates, show increased NaCl-tolerance, develop H_2S and produce spores the morphology of which is characteristic of this

Table 1

A comparison between our thermal-well Bacillus isolated and the standard description of Bacillus stearothermophilus given by CLAUS and BERKELEY (1986) furthermore GORDON, HAYNES and PANG (1973) on the basis of the most common differential-diagnostic features

Diagnostic features	Bacillus stearothermophilus	Representative Bacillus strains of thermal well No. II
Cell, diameter (μm)	1.0	0.75
Cell, length (μm)		3-6
Gram staining	v	v
Spore, shape	E	E
Spore, location	terminal or subterminal	
Deformed sporangia	+	
Active movement	+	+
Catalase test	a	+
Oxidase test	d	+
Oxidative decomposition of glucose		+
Fermentative decomposition of glucose		+
Growth under anaerobic conditions	-	a
Utilization of citrate	b	-
Utilization of propionic acid		b
Casein hydrolysis	a	+
Gelatin hydrolysis	+	a
Starch hydrolysis	+	+
Growth at pH 5.7	-	+
pH 6.8	+	+
3% NaCl		+
5% NaCl	b	+
7% NaCl	-	+
9% NaCl		+
H_2S production		+
Indol production	-	-
N_3H production		+
Voges-Proskauer test	-	-
pH in the VP-medium	6	6
Reduction of nitrate to nitrite	a	+
Phenylalanine deaminase	-	-
Decomposition of Tween-20		b
Tween-40		a
Tween-60		a
Tween-80		-
Urease	-	-
Methyleneblue reduction		-
Resistance against lysozyme	-	b
Tolerance to NaN_3	-	+
DNase activity		+
RNase activity		+
Tyrosine decomposition	-	-
Phosphatase activity		+
Temperature maximum for growth (°C)	70—75	80
Temperature minimum for growth (°C)	30—45	40
Acid production from L-arabinose	b	a
ribose		+

Table 1 (cont.)

Diagnostic features		Bacillus stearothermophilus	Representative Bacillus strains of thermal well No. II
Acid production from	xylose	a	+
	fructose		+
	glucose	+	+
	mannose		+
	lactose		-
	sucrose		+
	dextrin		+
	glycerol		+
	inositol		-
	mannitol	b	+
	sorbitol		-
	ethanol		b
	methanol		-
Utilization of C-sources	arabinose		-
	ribose		a
	xylose		+
	rhamnose		b
	fructose		+
	galactose		+
	glucose		+
	mannose		+
	lactose		-
	sucrose		a
	trechalose		a
	raffinose		b
	dextrin		+
	dulcitol		-
	glycerol		a
	inositol		-
	mannitol		+
	sorbitol		-
	ethanol		b
	methanol		a
Resistance to antibiotics and antibacterials			
	ampicillin		É
	carbenicillin		É
	chloramphenicol		MÉ
	chlortetracycline		MÉ
	clindamycin		MÉ
	erythromycin		MÉ
	gentamycin		R
	kanamycin		R
	lincomycin		MÉ
	nalidix acid		R
	neomycin		R
	nitrofurantoin		MÉ
	nystatin		R
	oleandomycin		MÉ
	oxacillin		É
	oxytetracycline		MÉ

Table 1 (cont.)

Diagnostic features	Bacillus stearothermophilus	Representative Bacillus strains of thermal well No. II
Resistance to antibiotics and antibacterials		
penicillin		É
polymyxin-B		R
spiramycin		R
sumetrolim		MÉ
superseptyl		R
tetracyclin		MÉ
vancomycin		MÉ

+: >85% of the strains showed resistance.
d: the percentage of resistant strains changed between 11 and 89.
a: the percentage of resistant strains changed between 50 and 84.
b: the percentage of resistant strains changed between 15 and 49.
-: the percentage of resistant strains changed between 0 and 14.
v: the feature is variable.

species. On the basis of the data in Table 1, however, it can be stated that our B. stearothermophilus isolates differ, in certain diagnostic features, from those of the standard description of the species. At present we suppose, that the homogeneous Bacillus population of the thermal well No. II of the Széchenyi bath detected by us represents a local variety of B. stearothermophilus.

Presumedly these thermophilic bacilli can colonize somewhere in the water conducting pipe system of well No. II solid surfaces, from which they can continuously contaminate the moving water. It would be an unrealisable task to trace back on the true population size of this Bacillus in the well only on the basis of the measured densities of its cell numbers in the outflowing well water. Further studies will be necessary to clarify the colonization strategy of this Bacillus in the underground system of thermal well No. II.

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THE RHIZOPLANE MICROBIOTA OF TOMATO (LYCOPERSICUM ESCULENTUM L.). I. NUMERICAL ANALYSIS OF BACTERIAL ROOT COMMUNITIES OF THE FIRST GROWTH STAGES

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From the rhizoplane of tomato (Lycopersicum esculentum) 92 strains were isolated and studied. Eighty-two strains (non-streptomycetes) were subjected to cluster analysis, based on similarity values of 171 coded characteristics. The identification work of the streptomycetes was carried out by the classical ISP-methods.

The dominant species delineated by cluster analysis were a type of nocardioform actinobacteria, members of the genera Curtobacterium, Pseudomonas, and Xanthomonas. Some strains in more or less subordinate populatory position were identified as Streptomyces spp., Bacillus spp., Micrococcus spp., Arthrobacter sp. and a few coryneform actinobacteria.

A great percentage of strains (not belonging to the family Streptomycetaceae) was motile, inorganic phosphatase, amylase, caseinase, gelatinase, lipase, DN-ase and RN-ase positive, and produced acid from several carbon sources. All strains were cellulase negative.

Introduction

Investigation of the association among higher organisms and prokaryotes is nowadays a very important research area of microbiology. The examination of interactions between plants and bacteria is of particular interest because of its connection to agriculture, soil productivity, etc. As an example we could note an early article of SCHNEIDER (1982), discussing the question of "disease suppressive soils" and giving the explanation, that this phenomenon is inherent in correlation between the plant's metabolism and soil microorganisms.

The analysis of the composition of microbial consortia that live in the rhizoplane, as well as the comprehension of their co-operation with the host-plant, plays a significant role in the perfection of chemical fertilization methods. Besides, the results of such investigations could also be used by phytophathologists for the biological control of plant diseases (HASEGAWA et al. 1990; KLOEPPER et al. 1991).

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Numerous reviews of past experiments show clearly the direction that the research on rhizosphere and rhizoplane microbiotas has taken in this century. In general, rhizosphere and rhizoplane have been characterized in four different manners: morphologically (CLARK 1940; WEBLEY et al. 1952; ROVIRA 1956); by physiological and biochemical characteristics (STARKEY 1931; LOCHHEAD 1940; KING and WALLACE 1956); through the nutritional requirements of plants (WALLACE and KING 1954; LOCHHEAD abd ROUATT 1955; GYLLENBERG 1957) and by the determination of root isolates (SPERBER and ROVIRA 1959; VÁGNEROVÁ et al. 1960a--b, SZABÓ 1974; YIP and WESTE 1985; MARTINEZ et al. 1989; RAOL 1991; GHULAM 1992).

Until recently, lots of bacterial and fungal genera have been reported from rhizosphere and rhizoplane of various plants. Unfortunately, data on frequency of incidence of different taxa are deficient and the taxonomic ranking of microbes is mostly dubious. In spite of the fact that tomato (Lycopersicum esculentum L.) is a very important vegetable crop, our knowledge on its microbiota is fairly insufficient. Owing to this reason, it was selected as an object of this research. In the present paper, an attempt is made to describe the root surface bacterial communities of the initial growth stages of tomato.

Material and Methods

I. Cultivation of tomato

The tomato sort, Lycopersicum esculentum "Kecskeméti merevszárú" was grown under conventional greenhouse conditions in 17 cm diameter plastic pots filled with "common black mould" (approx. pH 7; soil temp.: 20-25 ^{o}C). The seedlings were illuminated by the natural sunshine complemented daily with 4 hours of artificial light at 1000 lux intensity.

II. Sampling procedure

Five leafed stage tomato plants (approx. 8 week old) were carefully removed from the pots and freed of the bigger soil particles that adhered to the roots. Afterwards, under aseptic conditions, the roots were cut at the collar, the junction with the stem and placed in a beaker containing sterile water. Then with the help of sterile forceps, the roots were washed in autoclaved water by several changes. The roots cleaned in this way were put onto sterile filter paper and with forceps were freed of remaining big soil particles. Following this procedure the roots were repeatedly washed by shaking (15 times) aseptically in 250 ml flasks containing water. The whitened roots were dried with sterile filter paper and pulped in a sterile mortar.

III. Isolation of root surface bacteria

One g of the root pulp was serially diluted and spread on the surface of a) meat-peptone-yeast medium (peptone 5.0 g, beef extract 3.0 g, extract of yeast 1.0 g, agar 20.0 g, distilled water 1000.0 ml, pH 7.0, sterilization at 121 ^{o}C for 15') and b) starch-casein agar (starch 10.0 g, K_2HPO_4 0.5 g, casein 1.0 g, agar 20.0 g, distilled water 1000.0 ml, pH 7.0,

sterilization at 121 °C for 15'). The inoculated media were incubated at 28 °C for one week. The separated colonies were isolated without sorting onto slants of the same composition like the plating medium. 528 isolates obtained, were divided into groups according to their most notable macromorphological properties and 92 representative strains were selected. Among these, 10 were streptomyces. Pure cultures were made by repeated spreading and reisolation. The homogeneity of the strains was verified by phase-contrast microscopy.

Owing to unsatisfactory growth of strains, the isolatory media were changed for soybean agar as maintenance medium (soybean meal 20.0 g, starch 10.0 g, $CaCO_3$ 2.0 g, extract of yeast 5.0 g, NaCl 5.0 g, agar 20.0 g, distilled water 1000.0 ml, pH 7.0, sterilization at 121 °C for 15').

IV. Characterization and determination of strains

For the examination of Streptomyces strains the methods of SHIRLING and GOTTLIEB (1966) were adopted. The taxonomic identification was made according to the key of SZABÓ et al. (1975). Other bacteria (82 strains) were characterized by the methods outlined below (most of these were based on the procedures described by COWAN and STEEL 1974).

1. Colony morphology: soy-bean agar plates were spread by very diluted suspension of each strain and incubated at 28 °C for 5 days. Afterwards, the form, shape, surface and edge characteristics, consistency, height and pigmentation of colonies were examined. 2. Cell morphology and life cycle: the shape, dimensions and motility of cells were determined by microscopic examination of native preparations and Gram stained slides. The phase-contrast microscopic examination of life cycle was carried out with the help of slide cultures (under aseptic conditions, thin agar blocks were placed on slides, inoculated, covered with cover-slips and incubated). Observations were made every hour. The presence of flagella was determined by electron microscopy. Endospore formation was examined in malachite green stained preparations. 3. Temperature range of growth: growth of strains in soybean broth was observed at 4, 10, 37, 41 and 55 °C. 4. pH range of growth: the effect of pH values 3, 5.5, 7, 9 and 11 on the growth of our strains was investigated in meat-peptone-yeast extract broth. 5. Growth on Bacto MacConkey agar (Difco Manual 1984). 6. Tolerance to 5, 7 and 9% NaCl: in meat-peptone-yeast broth. 7. Anaerobic growth: Difco Tryptic Soy agar slant cultures were incubated for a week in Oxoid anaerobic box at 28 °C. 8. Catalase test. 9. Methylene blue reductase (GORDON and HAYNES 1973). 10. Nitrate reduction to NO_2^-, NH_4^+ and N_2. 11. Oxidation and fermentation of glucose. 12. Methyl red. 13. Acetoin production. 14. Indole formation. 15. Phosphatase test. 16. Oxidase. 17. Urease activity. 18. DNase and RNase tests (JEFFRIES et al. 1957). 19. Hydrogen sulphide production. 20. Gelatine hydrolysis. 21. Starch hydrolysis. 22. Digestion of casein. 23. Pigment formation in King's media (KING et al. 1954). 24. Growth in N free medium (LAPAGE et al. 1970). 25. Utilization of carbon sources (GORDON and HORAN 1968). 26. Utilization of nitrogen sources (SZABÓ 1974). 27. Production of acid from carbohydrates (GORDON and HAYNES 1973). 28. Utilization of citrate as sole source of C (Gordon and Mihm 1957). 29. Pigment formation in YDC agar (STOLP and GADKARI 1981). 30. Tween 80 hydrolysis. 31. Aesculin cleavage. 32. PHB production (STANIER et al. 1966). 33. Lysozyme resistance (BERD 1973). 34. Resistance to moist heat treatment at 45, 50, 60, 70 and 80 °C for 20 min. 35. Arginine dihydrolase (THORNLEY 1960). 36. Cellulase production: sterile filter paper strips were placed in the liquid medium recommended by GORDON and HORAN (1968) in 37. Ammonia production in peptone water. 38. Growth on Winogradsky's medium (WINOGRADSKY 1949). 39. TTC tolerance (BRADBURY 1984). 40. Sensitivity to antibiotics: nutrient agar plates were seeded with 24-28 h old suspensions of the strains, then paper disks containing antibiotics, produced by "Institute for Serobacteriological Production and Research, Human, Budapest" were placed on the surface of inoculated agar plates, which were incubated at 28 °C until growth was sufficient to locate zones of inhibition. The diameter of inhibition zones was measured in millimetres. 41. Detection of meso-DAP in the peptydoglican (TYIHÁK 1979, SZABÓ et al. 1990).

V. Numerical analyis

For the comparison of the character sets of our strains the Adansonian-type numerical taxonomy was used. Similarity measures were calculated according to the Sokal-Mitchener coefficient based on 171 coded characters, while clustering was done by the "complete linkage" method. The computing was run by a program of G. LŐRINCZ (1985).

Table 1

The diagnostic-key characters of Streptomyces strains isolated from the rhizoplane of tomato

Designation of strains	Media used	Sporangium morphology	Number of spores	Colour of aerial mycelium	Colour of substrate mycelium	Soluble pigment	pH sensitivity of substrate mycelium pigment		Spore surface morphology	Utilization of carbon compounds								
							HCl	NaOH		G	A	SH	X	I	M	F	RA	RF
	ISP-2	RF		GY(3fe)	Y-b(Co40)	-	-	-										
PA-96	ISP-3	RF		GY(3fe)	Y-b(Co37)	-	-	-		+	-	-	+	+	++	++	++	-
PA-97	ISP-4	RF	10–50	GY(3fe)	Y-b(Co38)	-	-	-	smooth	+	-	+	+	+	++	+	++	-
PA-98	ISP-6					-				+	-	-	+	+	++	+	++	-
	ISP-7																	
	ISP-2	RF		GY(g)	Y-b(Co37)	-	-	-										
	ISP-3	RF		GY(g)	Y-b(Co37)	-	-	-										
PA-99	ISP-4	RF	10–50	GY(g)	Y-b(Co39)	-	-	-	smooth	++	+	-	+	++	++	+	++	+
	ISP-6																	
	ISP-7																	
	ISP-2	RF		GY(3fe)	Y-b(Co81)	-	-	-										
	ISP-3	RF		GY(3fe)	Y-b(Co82)	-	-	-										
PA-100	ISP-4	RF	10–50	GY(3fe)	Y-b(Co82)	-	-	-	smooth	+	-	-	+	-	-	+	-	-
	ISP-6																	
	ISP-7																	
	ISP-2	RF		GY(5fe)	Y-b(Co88)	-	-	-										
	ISP-3	RF		GY(5fe)	Y-b(Co88)	-	-	-		+	+	+	+	-	-	+	++	+
PA-101	ISP-4	RF	10–50	GY(5fe)	Y-b(Co88)	-	-	-	smooth									
	ISP-6																	
	ISP-7																	
	ISP-2	S		Y(2ba)	Y-b(Co38)	-	-	-										
PA-102	ISP-3	S		Y(2ba)	Y-b(Co38)	Y	-	-		++	++	+	+	-	++	+	+	-
PA-103	ISP-4	S	10–50	Y(2ba)	Y-b(Co38)	B	-	-	smooth	+	++	++	+	-	+	++	+	-
PA-104	ISP-6									++	+	++	+	-	+	+	+	-
	ISP-7																	

	ISP-2	RA		GY(g)	Y-b(C28)	-	-	-										
	ISP-3	RA		GY(g)	Y-b(C28)	-	-	-										
PA-105	ISP-4	RA	10—50	GY(g)	Y-b(Co51)	-	-	-	hairy	+	+	-	+	+	++	++	++	-
	ISP-6																	
	ISP-7																	

ISP: International Streptomyces Project

RA: retinaculum-apertum
RF: rectus-flexibilis
S: spiral

GY: grey
Y: yellow
Y-b: yellow-brown
B: brown

G: D-glucose
X: D-xylose
F: D-fructose

A: L-arabinose
I: i-inositol
RA: L-rhamnose

SH: saccharose
M: D-mannitol
RF: raffinose

Results and Discussion

In general we can conclude from our results, that high percentage of strains (not belonging to the family Streptomycetaceae) was inorganic phosphatase, caseinase, amylase, gelatinase, DN-ase and RN-ase positive, and produced acid from several carbon sources. These results agree with the observations of other researchers (SATTAR and GAUR 1986; ROZYCKI 1986; LILJEROTH et al. 1991; KLOEPPER et al. 1991). Evidently, the possession of these characteristics by the rhizoplane microbiota benefits the host-plant (e.g. P supply). On the other hand all strains were cellulase negative. Undoubtedly, the presence of this metabolic activity on the root surface could be disadvantageous to the host plant. It is also worth nothing, that the majority of strains was motile. The motility helps the rhizoplane bacteria in the rapid colonization of the root surface by chemotactic response to root exudates.

I. The composition of the rhizoplane streptomycete population. The diagnostic properties of the 10 selected representative strains ordered according to their designations are presented in Table 1. The identification work was carried out on the basis of the ISP-species descriptions (SHIRLING and GOTTLIEB 1966) using the works of SZABÓ et al. (1975), TRESNER-BACKUS (1963) and PRAUSER (1964). The results were confirmed according to the comprehensive work of WILLIAMS et al. (1989) too.

Strains designated as PA-96, 97 and 98, were identified as *Streptomyces lipmannii*. PA-99 is identical to *Streptomyces spadicis*, PA-100 resembles *Streptomyces aburaviensis* and PA-101 *Streptomyces alvidus*. Strains PA-102—104 were considered as *Streptomyces flavidovirens (aurigineus)* and PA-105 showed close similarity to *Streptomyces flaveolus*.

The incidence of the streptomycetes in the rhizoplane has also been demonstrated by other researches (SZABÓ 1974; SZABÓ 1989; GHULAM 1992, etc.). In most cases, only a few species could be isolated. According to the results, it is obvious that each plant selects different species from the soil streptomycete population, but the frequency of Streptomycetes in rhizoplanes seems to be relatively low, compared to other bacteria.

II. The result of the cluster analysis. Eighty-two strains were submitted to cluster analysis. The resulting dendogram is presented in Fig. 1. The similarity tree clearly shows that the strains segregated into 12 groups and a few isolated bacteria as single strain phenons. For the taxonomic identification of the strains, the works of BALOWS et al. (1992), GOODFELLOW
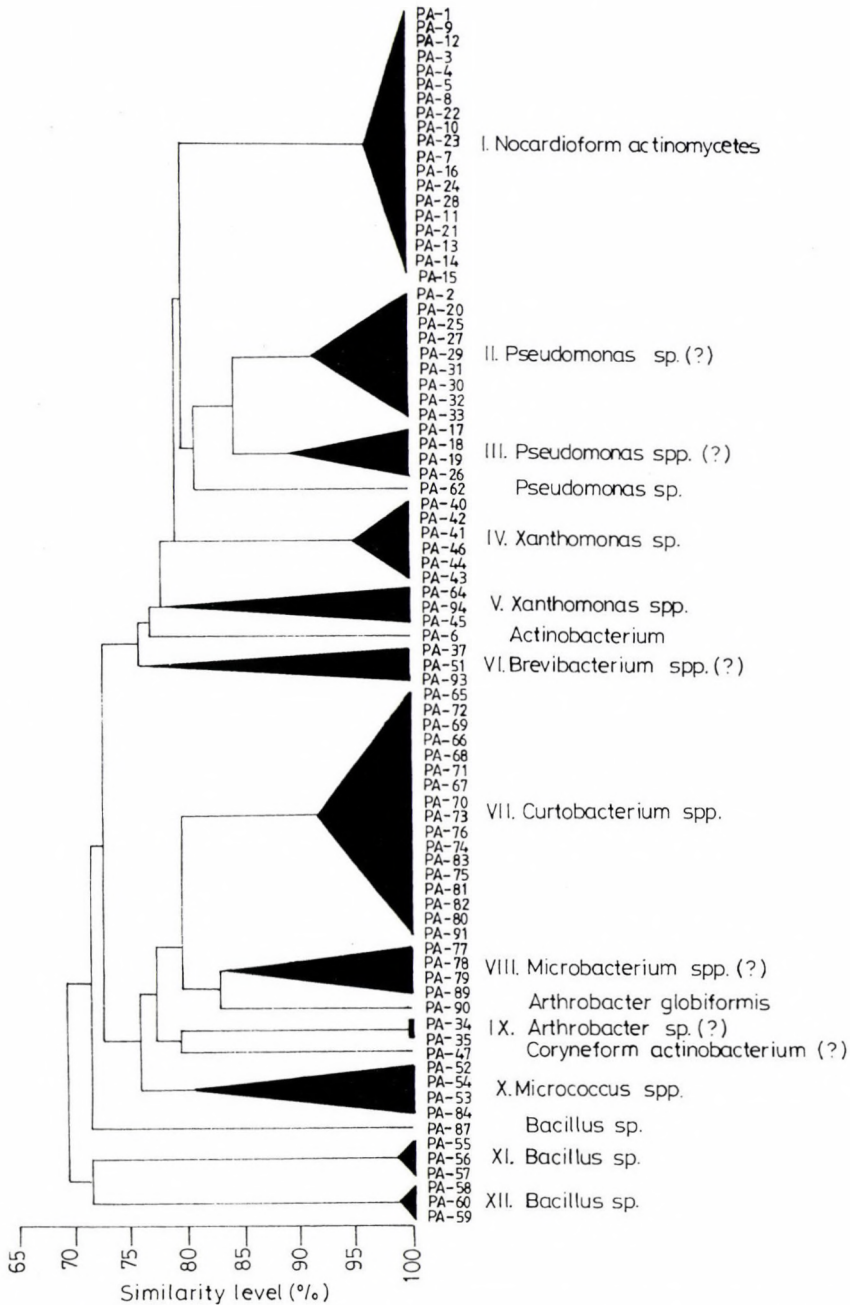
Fig. 1. Dendogram elaborated on the basis of 171 diagnostic key-characteristics of 82 bacteria isolated from the rhizoplane of tomato (*Lycopersicum esculentum*)
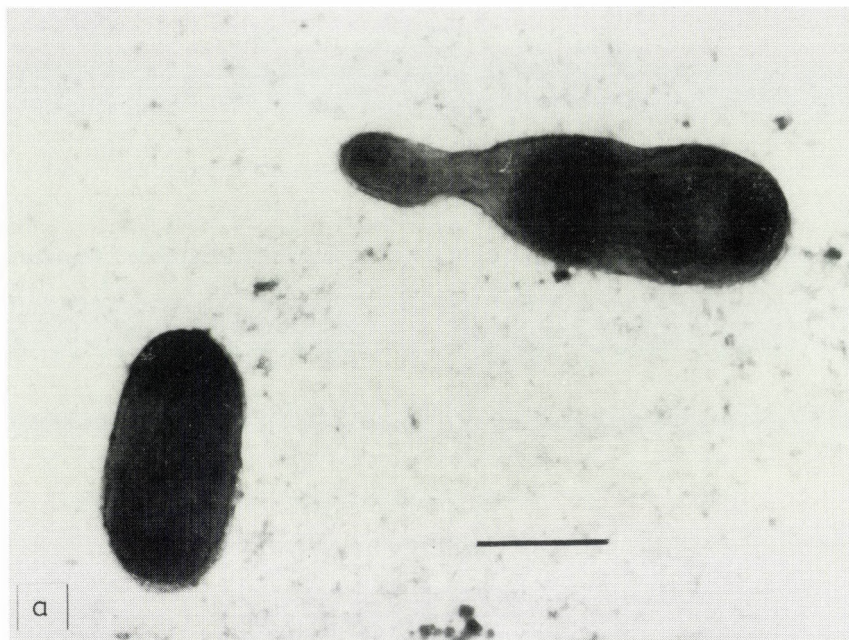
Fig. 2. Electron micrographs showing the life cycle of group I strains grown on soybean medium at 28 ^{0}C. Inoculum coccoid cells as in (e); (a) 4 h, (b) 6 h, (c) 12 h, (d) 16 h and (e) 24 h, old cultures. Bar = 1.0 μm

Fig. 2b.

Fig. 2c.

Fig. 2d.
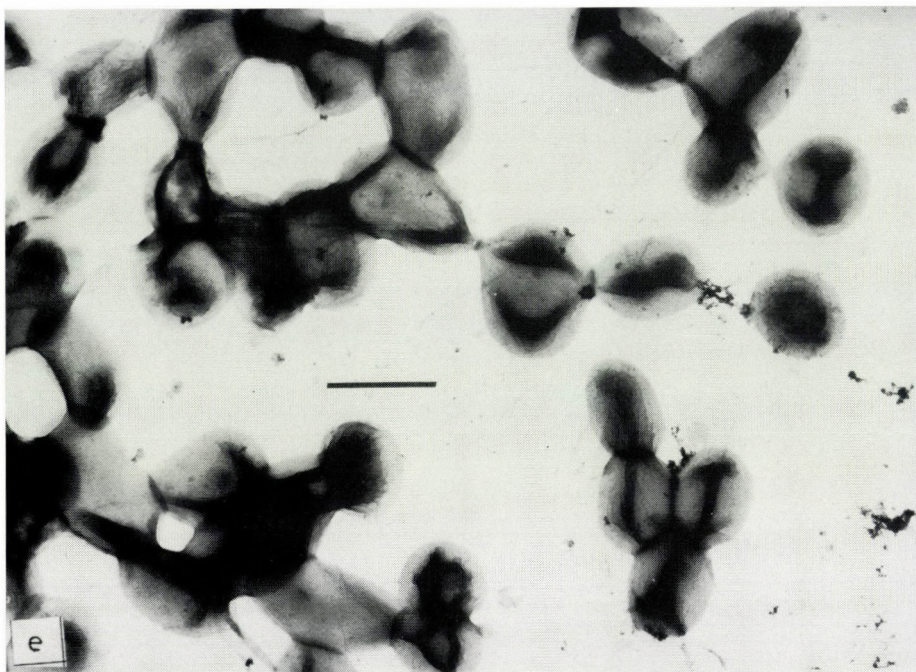
Fig. 2e.

et al. (1984), KRIEG et al. (1984), SNEATH et al. (1986), STARR et al. (1981) and WILLIAMS et al. (1989) were used.

Group I. This group has been created from 19 representative strains. The computer united them at 96% similarity level. They were facultatively anaerobic, non-endospore forming, non acid-fast, non-motile, pleomorphic, Gram positive bacilli, that showed a complicated life-cycle (Fig. 2). In soybean agar the diameters of their hyphal filaments were between 1.0 and 2.0 µm and they did not form aerial mycelium. Their cell wall contains meso-DAP and negative results were recorded in oxidase and nitrate reduction tests. The members of this group, according to their other important diagnostic-key characters (Table 2) could be identified as nocardioform actinomycetes, but we could not determine them as members of known taxa.

From the rhizoplane of several plants, other research workers have isolated members of the genus Nocardia, which could not be exactly identified (SPERBER and ROVIRA 1959). These bacteria were defined as Nocardia-like arthrobacters but very few of their key-characteristics were published. In my opinion their precise taxonomic identification requires detailed chemotaxonomic analyses (e.g. fatty acid profile determination).

Table 2

Some important physiological and biochemical properties of bacteria of groups I and II isolated from rhizoplane of tomato

Properties	Designation of groups	
	I	II
Aerial mycelium in soybean agar	-	-
Branching vegetative mycelium in soybean agar	+	-
Spore formation	-	-
Soluble pigment in soybean agar	brown	-
Insoluble pigment in soybean agar	beige	yellow
Gram staining	+	-
Motility	-	+
Anaerobic growth	+	-
Elevation of colonies on soybean agar	high	flat
Edge of colonies on soybean agar	undulate	entire
Growth on N-free medium	+	-
Growth on Winogradsky's medium	+	-
MacConkey agar	+	+
Tolerance to NaCl		
5%	+	+
7%	-	-
9%	-	-
pH tolerance:		
3	-	-
5.5	+	+
7	+	+
9	+	+
11	-	-
0.01% lysozyne tolerance	+	+
Growth at:		
4 ^{o}C	+	+
10 ^{o}C	+	+
16 ^{o}C	+	+
26 ^{o}C	+	+
37 ^{o}C	+	+
41 ^{o}C	-	-
Moist heat resistance		
50 ^{o}C/10 min	+	+
60 ^{o}C/10 min	+	-
70 ^{o}C/10 min	-	-
Oxidase reaction	-	+
Catalase production	+	+
Nitrate reduction		
to NO_2^-	-	-
to NH_4^-	-	-
to nitrogen	-	-

Table 2 (cont.)

Properties	Designation of groups	
	I	II
Hydrolysis of:		
urea	-	±
Tween 80	+	+
aesculin	-	-
starch	-	-
gelatin	-	d
casein	-	D
Arginine dihydrolase	-	-
Phosphatase	-	+
Growth on Simmon's citrate medium	-	-
Acetoin production	-	-
Methyl red	-	-
Methylene blue reduction	-	+
Cellulase production in synthetic medium	-	-
PHB formation	+	+
H_2S from peptone	-	D
DN-ase	+	d
RN-ase	+	+
Lysine decarboxylase	-	-
Glucose breakdown (Hugh-Leifson)		
oxidative	+	+
fermentative	+	-
Utilization of:		
D-glucose	-	-
L-arabinose	-	-
Sucrose	-	-
D-xylose	-	-
Mezo-inositol	-	-
D-mannitol	-	-
Raffinose	-	-
Galactose	+	-
Lactose	-	-
Mannose	+	-
D-ribose	+	-
D-trehalose	-	-
Glycerol	-	-
D-sorbitol	-	-
Dextrin	-	-
D-fructose	-	-
Ethanol	+	-
Dulcitol	-	-
L-rhamnose	-	-
Acid production from:		
Mezo-inositol	-	+
D-mannitol	+	+
D-sorbitol	-	-
Dulcitol	-	d
Glycerol	-	-
Ethanol	+	+

Table 2 (cont.)

Properties	Designation of groups	
	I	II
Acid production from:		
Inulin	+	+
L-arabinose	+	+
D-trehalose	-	+
D-fructose	+	+
Raffinose	+	+
Melezitose	-	d
Lactose	-	+
D-glucose	+	+
Galactose	+	d
Salicin	-	d
D-xylose	-	d
L-rhamnose	-	+
Sucrose	-	d
D-mannose	+	-
D-ribose	+	-
Dextrin	-	-
Utilization of N sources:		
$Ca(NO_3)_2$	-	-
$NaNO_3$	-	-
KNO_3	-	-
NH_4NO_3	-	-
$(NH_4)_2CO_3$	-	-
$(NH_4)HPO_4$	-	-
$(NH_4)_2SO_4$	-	-
NH_4Cl	-	-
L-cysteine	+	+
L-asparagine	+	+
L-arginine	-	-
L-glycine	-	d
L-tryptophan	-	D

Symbols: +: 90% or more of the strains are positive,
-: 90% or more of the strains are negative,
d: 50—89% of strains are positive,
D: 51—89% of strains are negative,
±: indefinite.

Group II. Consists of 9 strains. They were amalgamated by the computer at approximately 90% similarity value. They were obligatory aerobic, non-endospore forming. Gram negative, straight or slightly curved rods, 0.8-1.0 µm in diameter by 1.0-5.0 µm in length, motile by single polar flagellum (Fig. 3), and their colonies exhibited yellow pigmentation. Oxidase and catalase tests showed positive results. Negative results were recorded in arginine dihydrolase, lysine decarboxilase and nitrate reduction tests. On
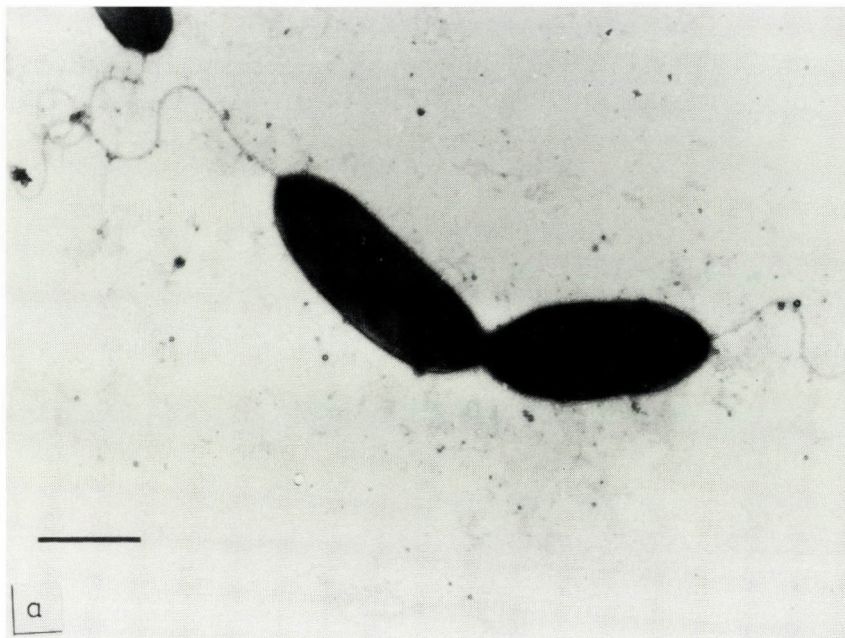
Fig. 3. Electron micrographs of strains in the groups II—III, showing single polar flagellation in 24 h old cultures: (a) PA-30, (b) PA-17, (c) PA-19. Bar = 1.0 μm

Fig. 3b.
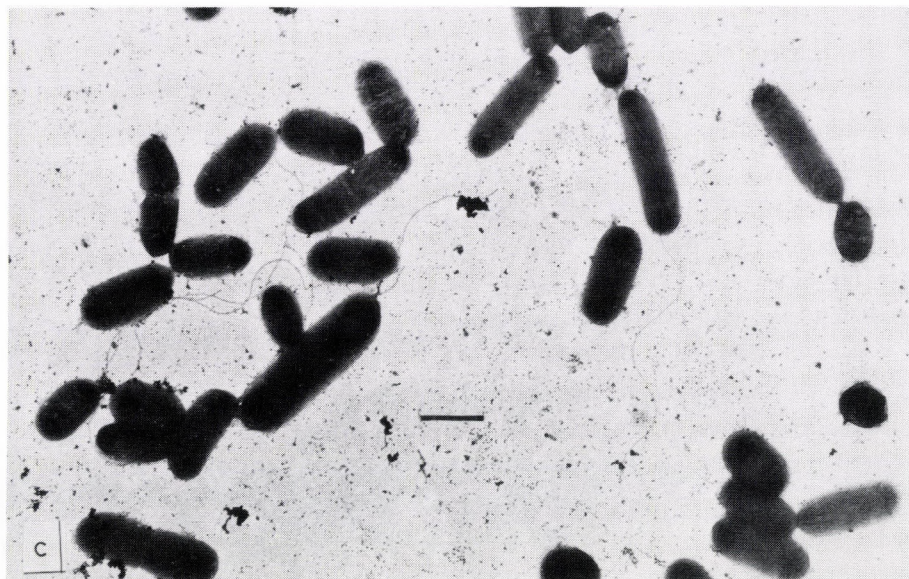
Fig. 3c.

the basis of their diagnostic characteristics (Table 2), these bacteria belong to the family Pseudomonadaceae and probably are members of the genus Pseudomonas in spite of the fact that their cell wall did not contain meso-DAP and they did not utilize any carbon sources if yeast extract was not present in the media.

Group III. Four strains were included in this group, having an average similarity level of 87%. Their morphological, physiological and biochemical characteristics are similar to those of the former group, showing a phenetic similarity of approximately 83%. This group can be divided into two subgroups. Stains designated as PA-17 and -18, were united at 100% similarity level, grew at pH 3 and did not show resistance to 60 ^{0}C moist heat treatment for 10 min (subgroup IIIa). The members of the subgroup IIIb (PA-19 and PA-26) were combined at 93% similarity value. Both subgroups belong to the genus Pseudomonas.

The strain designated as PA-62 is considered as a member of the genus Pseudomonas, but we could not identify at species level.

Although Pseudomonas spp. are frequent members of rhizoplane bacterial communities, the identification of supposed pseudomonas isolated from the rhizoplane of different plants was usually not exhaustive (SPERBER and ROVIRA 1959; VÁGNEROVÁ et al. 1960a). But it is a well-known fact that some

bacteria isolated from rhizoplane need growth factors or are characterized by a negative reaction to most of physiological tests (LILJEROTH et al. 1991, cit. CURL and TRUELOVE 1986). In relation to the absence of meso-DAP, in the peptydoglican of Pseudomonas, the available data are unsatisfactory.

Group IV. This phenon consists of six stains united at 95%. According to their diagnostic key-characteristics examined, this group was identified as member of the genus Xanthomonas.

Group V. Harbours three strains, which were united at 77% similarity value. Their differential characteristics were similar to those of the former group. They were identified also as members of the genus Xanthomonas. In spite of the fact that plant diseases caused by Xanthomonas species occur all over the world, some researchers mention the occurrence of phytophathogenic xanthomonads in asymptomatic non host plants or on host plants, as non-pathogenic epiphytes, and in infected but presymptomatic host plants (cit. STARR et al. 1981; SPERBER and ROVIRA 1959). Further investigations are needed to elucidate the plant pathogenic behaviour of our strains.

On the basis of its most important diagnostic characters and its life-cycle, the strain designated as PA-6 was identified as member of the group Actinobacteria. (According to GOODFELLOW et al. 1984.)

Group VI. This small group was created from three coryneform strains. Each one could multiply only under obligatory aerobic conditions, forming branching filaments in the medium, with a characteristic non soluble yellow pigment production. Some of their key-characters (e.g. peptidoglycan contains meso-DAP, life-cycle, obligatory aerobic, catalase positive) show resemblance to the key-characteristics of the genus Brevibacterium, however, they have single polar flagellation.

Members of the genus Brevibacterium have been isolated from the rhizoplane of other plants like subterranean clover, rye-grass and black locust (SPERBER and ROVIRA 1959; SZABÓ 1974). Chemotaxonomical studies over the past 20 years have confirmed the extreme heterogeneity of the genus and resulted in the classification of many Brevibacterium species into other coryneform genera (including Arthrobacter, Aureobacterium, Corynebacterium, Curtobacterium, Microbacterium, Nocardioides, Oerskovia and Rhodococcus (cit. BALOWS et al. 1992). Our strain seem to be true members of the genus.

Group VII. Seventeen strains were included in this phenon, with 91% similarity. They were identified as members of the genus Curtobacterium. Most of the members of the genus Curtobacterium have been isolated from plants. Curtobacterium citreum, Curtobacterium albidum and Curtobacterium luteum

were isolated from rice. The phytopathogenecity of these species is not known. C. flaccumfaciens is however the only species regarded as a plant pathogen (cit. SNEATH et al. 1986). Our strains could not be identified at species level.

Group VIII. Four strains were united at 83% similarity value in this group. They seem to belong to the genus Microbacterium although, in many characteristics they differ from the type species. Further biochemical studies will be necessary to confirm this opinion. In spite of the fact that the soil usually, is not habitat of Microbacterium. TOPPING reported the isolation of Microbacterium liquefaciens from it. ABD-EL-MALEk and GIBSON pointed out that her M. liquefaciens group possessed extremely variable characteristics (cit. STARR et al. 1981).

The strain designated as PA-90 was identified as member of the "group" Arthrobacter globiformis.

Group IX. Two identical strains (obligatory aerobic, Arthrobacter type life-cycle, non acid fast, catalase positive) that show resemblance to the genus Arthrobacter, Rhizosphere is the usual habitat of Arthrobacter and some researchers isolated members of this genus from the rhizoplane of several plants like tobacco, subterranean clover, rye-grass and wheat (SGUROS 1955; SPERBER and ROVIRA 1959; VÁGNEROVÁ et al. 1960a).

The strain designated as PA-47 shows resemblance to non-branching filament forming coryneform bacteria isolated by ZALMUN (1993) from the Danube. We could not identify this even at genus level.

Group X. This small group consists of four strains. Three of them, designated as PA-52, 53 and 54, were united at approximately 98% similarity level and were identified as Micrococcus rosens. The other strain PA-84 proved to be taxonimically similar to the species Micrococcus varians. In many instances, members of genus Micrococcus were isolated from the rhizoplane of several plants (VÁGNEROVÁ et al. 1960a; SZABÓ 1989). Worthy of mention that our strains are inorganic phosphatase positive, contrary to the typical genus characteristic. According to SZABÓ (1992), KEKHAJOVA and TALEVA (1990) this phenomenon is common in the rhizosphere, because this property favours the host-plant.

The strain PA-87 and the members of small groups designated as XI and XII are all endospore forming, catalase positive, motile, Gram positive bacteria that were identified as members of the genus Bacillus, but were not determined at species level. Many times, members of this genus were isolated from the rhizoplane (SPERBER and ROVIRA 1959; VÁGNEROVÁ et al. 1960a, cit.

SZABÓ et al. 1989), but they could not be exactly identified. Usually the incidence rate of Bacillus in the rhizoplane is very low. According to SZABÓ (1989) the root surface is not ideal habitat to members of this genus.

Our results obtained agree with the outcomes of many previous investigations. Gram negative rods and actinobacteria were usually predominant in the rhizoplane, while the incidence of streptomycetes, endospore forming, Gram positive bacilli and cocci were low (VÁGNEROVÁ et al. 1960b; CAMPBELL 1985; SZABÓ 1989).

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COMPARATIVE TOXICITY OF TWO PESTICIDES TO A GREEN ALGA CHLORELLA VULGARIS

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The effects of two pesticides, an organophosphorus (dimethoate) and an organochlorine (endosulfan) on Chlorella vulgaris have been evaluated and compared taking the rates of survival, optical density of the culture, log cell number and chlorophyll biomass as parameters. No reduction of survivability of the alga was found at concentrations $\leq$ 1.0 mg/L of both the pesticides tested separately: on the other hand, there was little survival beyond 100 mg/L of dimethoate and 75 mg/L of endosulfan. In all cases the former was less toxic than the latter at any of the concentrations tested. With an exception of growth stimulation initially at the low doses, the chemicals acted adversely and differentially on growth of the alga. The LC_{50} values were 51.0 $\pm$ 0.79 mg/L of dimethoate and 41.5 $\pm$ 1.39 mg/L of endosulfan while the static concentrations of the chemicals were 102.1 $\pm$ 1.17 mg/L and 78.6 $\pm$ 1.78 mg/L, respectively. Neuman—Keul's and Dunnett's tests revealed a positive correlation between the lowest effective concentration of dimethoate and the age of the culture while a negative in case of endosulfan. Reduction in growth and pigment content of C. vulgaris was time as well as dose dependent in all cases. Both the pesticides were, however, detoxified partially through completely by the alga tested even with higher concentrations, though dimethoate detoxification rate was more than the other. The initial cell density regulated the toxicity effects of both the chemicals, the former directly relating the lowest effective concentrations and the static dose.

Introduction

The aquatic ecosystem is an aqueous medium containing a large number of interacting physico-chemical parameters with organic and inorganic compounds in dissolved or suspended state and sustains a variety of plant and animal life. All these factors exist in a dynamic equilibrium in a fixed space and time. Water bodies, owing to the presence of aerobic autotrophic organisms, have been endowed with a remarkable capacity to revive their vitality. However, with an increase in the human population, the use of pesticides to boost agricultural production has caused the contamination of water bodies with such chemicals. This has changed the physical, chemical and biological picture of water bodies affecting water uses consequently.

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Besides, the concentration of these pollutants accumulates through run-off waters in fresh water bodies and produces harmful effects on the microorganisms which, however, are not target of these chemicals. Even at very low concentrations, the non-target organisms are generally succeptible to such toxicants. Thus when any pesticide enters into an ecosystem it may selectively remove some succeptible species from the range of the organisms present. This obviously leads to a reduction in species diversity and a change in the community structure.

In addition to direct toxic effects of the pesticides on phytoplanktons, other important secondary effects usually are resulted. Selective removal or alteration of a population of phytoplankton brings in modification of the food web. This causes a change in energy and matter flow patterns. Thus ecosystem characteristics such as total respiration, primary production, respiration to primary production ratio, nutrient cycling rates, etc. tend to change even momentously. It is, therefore, interesting and necessary to predict specific effects of toxicants on succeptible organisms like phytoplankton.

Both organophosphorus and organochlorine pesticides are, at present, used for the control of pests in agricultural practices. These two groups are toxic to algae though the toxicity is concentration dependent (MALY and RUBER 1983; SAROJA-SUBBARAJ and BOSE 1984; MEGHARAJ et al. 1987; ADHIKARY 1989; MOHAPATRA and MOHANTY 1992). The toxic effect of these pesticides on the microalgae results change in their diversity pattern (MALY and RUBER 1983; MEGHARAJ et al. 1987), growth rate (ADHIKARY 1989), growth pattern (MOHAPATRA and MOHANTY 1992) and photosynthetic efficiency (SAROJA-SUBBARAJ and BOSE 1984). The present paper deals with a comparative account of toxicity of two pesticides viz. dimethoate (organophosphorus) and endosulfan (organochlorine) to a planktonic green alga Chlorella vulgaris. It also deals with the change in toxicity effect of the pesticides in response to initial cell density and the capability of the alga to detoxicate the pesticides.

Material and Methods

The axenic culture of C. vulgaris Beij. was grown in modified Chu 10^+ medium (SAFFERMAN and MORRIS 1964) containing A_6 micronutrients of GERLOFF et al. (1950). The cultures were maintanied in a culture room illuminated with day light fluorescent tubes light/dark cycle 12/12 h PPFD 70 μmol/m^2 sec, PAR (400—700 nm), RH 75%. The stock and experimental cultures were done in non-absorbent cotton stoppered 250-mL and 100-mL borosilicate glass conical

flasks containing 100 mL and 25 mL of cultures, respectively. The stock cultures were transferred into fresh medium and every 7 days to keep the cells at their exponential phase of growth. The cultures of 7 days were used as initial inoculum for the experiments.

The test chemicals were an organophosphorus pesticide, dimethoate /O, O-dimethyl-S-(-N-methyl-carbamoylethyl)-dithiophosphate/ and an organochlorine pesticide, endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6,9-methano-2,4,3-benzodioxanthiepin-3-oxide), obtained from Rallis India Ltd., Bombay. Stock solutions (1 g/L) of the pesticides were prepared in the strile Chu No$^+$ medium under aseptic conditions for repeated use. These solutions were diluted to prepare different concentrations in the experimental cultures after the lag phase.

The measurement of survival was done by applying different concentrations of the pesticides separately on the nutrient agar plates (borosilicate glass plates with 10 cm diameter) and allowing the algal colonies to grow on them. The stock culture, prior to its use, was diluted aseptically with sterile Chu No$^+$ medium to get 1.2×10^4 cells/mL and from this, a sample of 0.1 mL was evenly spread on each plate under sterile condition. The cultures were incubated for 10 days and the rate of survival was calculated by counting the colonies and considering the survival in the control as 100%. The LC_{50} and static concentrations were then calculated at 95% confidence interval (SNEDECOR and COCHRAN 1967). The measurement of the toxicity effect of the pesticide on the alga was done by observing the change in log cell number (SOROKIN 1973), optical density (O.D at 660 nm) and pigment biomass (ARNON 1949) for 16 days at 4 days interval.

Three higher concentrations of both dimethoate (75, 100 and 125 mg/L) and endosulfan (20, 30 and 40 mg/L) were used in the detoxification study. Three sets of cultures, each in triplicate, were incubated at these concentrations for one week in 100-mL borosilicate glass conical flasks containing 25 mL of medium. The O.D. of the homogenized cultures of one set was taken after 7 days while the replacement of the organisms, which was done after centrifugation under aseptic condition at 3000 rpm for 10 minutes and reinoculum with equal sized inoculum (1.40×10^6 cells/mL), for the other two sets was made at the week end. This was repeated for another week to assess the rate of detoxification.

The toxicity effect of the pesticides at different cell density level of the alga was studied taking three different sized initial inocula (0.35×10^6, 1.40×10^6 and 2.45×10^6 cells/mL at initial level). The cultures were grown in borocilicate glass tubes (18 x 150 mm) each containing 10 mL of medium. Three different concentrations (lowest inhibitory at low cell density, LC_{50} and static) of both the pesticides were selected from the survival curve and were taken in these tubes. The tubes were incubated under same culture conditions for 8 days and observation was made at every 2 days by measuring the log cell number and OD of the homogenised cultures. The three cell densities have been referred as LCD (Low cell density), MCD (medium cell density) and HCD (high cell density) in the text.

The lowest effective concentrations, standard errors of the triplicates, LC_{50} and static concentrations, confidence intervals and lowest significant deviations (LSDs) were calculated statistically for interpretation of the results (SNEDECOR and COCHRAN 1967). Newman—Keul's test and Dunnet's test (ZAR 1984) were followed to calculate the relation between age of the culture and inhibitory action of the pesticides and to compare the action of one with the other.

Results and Discussion

Both the pesticides at concentrations $\leq$ 1.0 mg/L caused no reduction of survival of C. vulgaris while at 10 mg/L significant inhibition were recorded (7% and 15.6% of the control in case of dimethoate and endosulfan, respectively). The survival values at different concentrations of each of the pesticides were significantly different from each other except at their
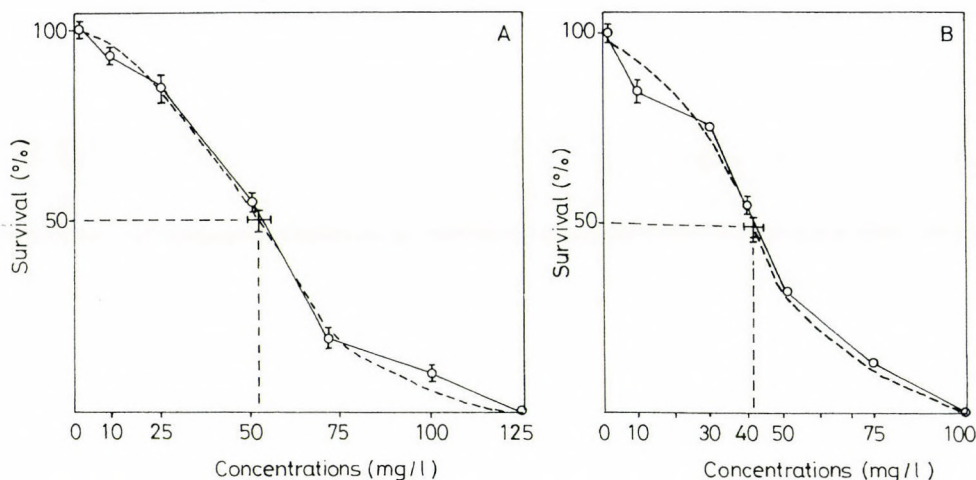
Fig. 1. Effect of (A) dimethoate and (B) endosulfan on the survival rate of C. vulgaris

low concentration(s) ($\leq$ 1.0 mg/L) which did not significantly differ from that at the control (Fig. 1). The 50% survival of the alga was observed at 51.0 $\pm$ 0.79 mg/L of dimethoate and 41.5 $\pm$ 1.39 mg/L of endosulfan (at 95% confidence limit) indicating these doses as sublethal (LC_{50}) to the alga. The growth of the colony remained static at 102.1 $\pm$ 1.17 and 78.6 $\pm$ 1.78 mg/L of dimethoate and endosulfan, respectively ($p = 0.05$). The algal colonies fail to grow on the agar plates at the presence of 125 mg/L of dimethoate and 100 mg/L endosulfan due to complete death of algal cells at the initial stage of incubation. At any rate, survival rate was more affected by endosulfan than dimethoate at any of the concentrations tested. Both the static and cidal doses of endosulfan were, however, less than those of dimethoate supporting the view that organochlorine pesticides are more toxic than organophosphorus pesticides (ANTUNES-MADEIRA et al. 1980; ADHIKARY 1989; MOHAPATRA and MOHANTY 1992). The inhibitory effect of both the pesticides on the development of colony of C. vulgaris on the agar plates is due to the cell destruction caused during exposure (ANTUNES-MADEIRA et al. 1980; NETRAWALI and GANDHI 1990).

Figure 2 contains the growth curves, based on the mean cell counts of the cultures treated with different concentrations of the pepticides. In case of dimethoate the log cell number at 1 mg/L did not vary significantly from the control on the first observation (4th day) though it significantly increased even at this concentration on the subsequent observations. At all
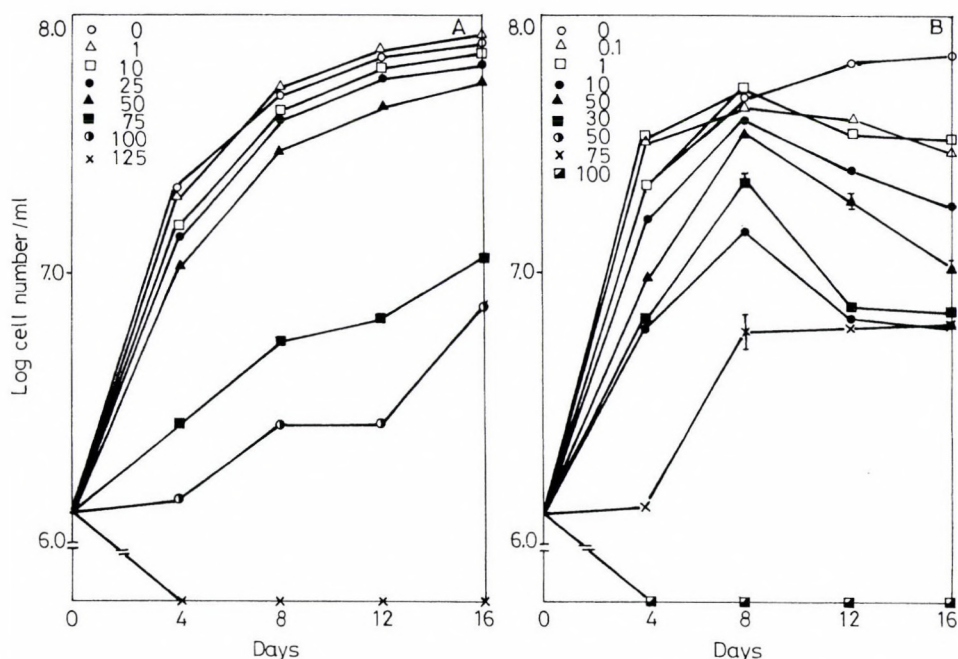
Fig. 2. Effect of (A) dimethoate and (B) endosulfan concentration (mg/l) on the mean cell count of C. vulgaris

other tested concentrations (≥ 10 mg/L ≤ 125 mg/L) of the pesticide the mean values of log cell number were always significantly different from one another. However, at 10 mg/L of the pesticide compared to the control the inhibitory effect was significant, up to 8th day after which the cell numbers tended to that in the control. The initial cell densitiy more or less remained constant up to the 12th day at 100 mg/L of dimethoate and a slight increase, though significant, was observed thereafter.

The change in the cell density vis-à-vis mean values of the log cell number of cultures treated with different concentrations of endosulfan was remarkably different from that of dimethoate toxicity (Fig. 2B). At 0.1 and 1 mg/L of the pesticide increased values of the log cell number were recorded till the 8th day while it was reversed thereafter. While the acceleration of log cell number was significant at these two concentrations on the 4th day, it was not so on the 8th day. Though the toxic effect of the pesticide was significant at the higher concentrations, it was not so up to 10 mg/L of the chemical on the 4th day. On the other hand, the inhibition of log cell number was significant at all the tested concentrations on the 12th and 16th day.
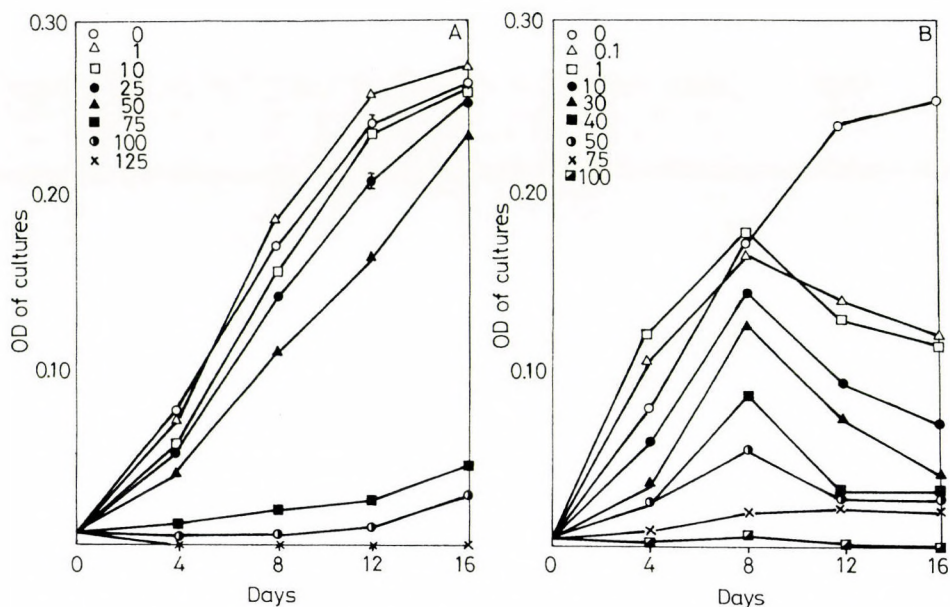
Fig. 3. Effect of (A) dimethoate and (B) endosulfan concentrations (mg/l) on the growth (O.D.) of C. vulgaris

Both the pesticides at all the concentrations tested were found to have some effect on the growth rate of C. vulgaris measured in terms of optical density of the homogenized cultures (Fig. 3). Acceleration of growth of the alga was observed at 1 mg/L of dimethoate, and 0.1 and 1 mg/L of endosulfan. However, the growth was continuously accelerated till the 16th day in case of dimethoate while it was found to be inhibited after the 8th day in case of endosulfan. Similarly, while the growth acceleration at 1 mg/L of dimethoate was insignificant at the first observation, it was found significant on subsequent observations. But at the presence of 0.1 as well as 1 mg/L of endosulfan the growth acceleration was significant on the first, not so on the second observation while growth was significantly inhibited on the subsequent observations.

The differences in the toxicity effect of the two pesticides were obvious at their higher concentrations. At 10 mg/L of dimethoate, for example, inhibition of growth was found to be more at the initial stage of the incubation (till 8th day) though it was comparatively reduced thereafter. In contrast, the inhibitory effect of endosulfan at the same concentration was

insignificant at the initial stage and was severe thereafter. The difference in the type of growth during the exposure period was also found at the static concentrations of the pesticides. While in case of dimethoate remarkable increase in O.D., compared to the O.D. at the initial stage, was observed after 11 days of incubation, slight increase of O.D. on the 8th day and decrease thereafter was observed at the presence of endosulfan.

The growth curves, based on the mean cell counts at each concentration of the pesticides in every observations were used for further statistical analysis. The areas under these growth curves, drawn by plotting the mean log cell count values against age of cultures in each pesticide were compared for each alga using the Newman—Keul's test (ZAR 1984). The areas under the growth slopes of each observation were also compared separately to find out the pattern of changes in the degree of significance of inhibitory action of the pesticides with prolongation of exposure time. The test showed that compared to control, growth was significantly accelerated at 1 mg/L of dimethoate but, on the other hand, the growth inhibitions at all other tested concentrations were significant ($p = 0.05$). Also in case of endosulfan there was significant (inhibition) difference ($p = 0.05$) between the growth over the culture period in any of the tested concentration of the pesticide. However, taking only first two observations it was observed that the growth acceleration at 0.1 and 1 mg/L of endosulfan caused significant increase in area under the growth curve compared to that of the control.

Dunnett's test was used to calculate the lowest effective concentration (LEC) of each pesticide where significant inhibition of growth was observed using the replicate cell counts for each chemical, at each concentration, the areas under growth curves over different culture periods were calculated. The areas for each treatment level were compared to the control (ZAR 1984) to find out the lowest effective concentrations ($p = 0.05$). Observation showed that the LEC varied not only with the pesticides but also with the incubation period. The LEC values of dimethoate were 2.04 mg/L, 2.79 mg/L, 3.15 mg/L and 4.69 mg/L on the first to last day of observation, respectively while, on the other hand, such respective values were 1.68 mg/L, 1.12 mg/L, 0.11 mg/L and 0.09 mg/L in case of endosulfan.

C. vulgaris showed a wide range of tolerance to both the pesticides. The toxicity effects of the chemicals to the alga are quite different from each other and dependent on both concentrations as well as age of the cultures. Dimethoate was found to be growth stimulatory at 1 mg/L like other organophosphorus pesticides (MEGHARAJ et al. 1987). The increase in the LEC

value of dimethoate corresponding with the age of the cultures shows the time dependent reduction of toxification which may either be due to biodegradation or adaptation of the species to the treated medium over the course of the experiment. Organophosphorus pesticides, at higher concentrations, induce lesions in the cell membrane and their enlargement in the post-treatment period due to their interaction with lipid bilayer causing disordering effects (ANTUNES-MADEIRA et al. 1980; NETRAWALI and GANDHI 1990). The increased disorder in the hydrophobic core can weaken the interactions of the fatty acid chains by Van der Waal attractions (LENAZ 1979) and decrease lipid adhesion. Such action on the cell membrane may cause physical and physiological changes which alter the native properties of the membranes (LINDEN et al. 1973; LEE and WILKINSON 1973; PHILLIPS et al. 1975; ANTUNES-MADEIRA and VICTOR-MADEIRA 1979) affecting membrane permeability, enzymes carrier activities and membrane fusion processes (SHIMSHICK and McCONNEL 1973; PHILLIPS et al. 1975; PAPAHADJOPOULOS and PORTIS 1978; MARCELJA and WOLFE 1979). These disorders could be reflected in the form of retardation growth which was observed in the present experiment. The increase in the LCE value and the toxicity reduction effect of higher concentrations of dimethoate with increase in the age of the culture is probably due to biodegradation and/or autodegradation of the pesticide and repair of the lesions on the membrane by the way of adaptation.

Endosulfan, on the other hand, showed a delayed effect on C. vulgaris at all the concentrations tested. It is known that C. vulgaris is an efficient accumulator of organochlorine pesticides (HANSEN 1980). The uptake and accumulation of these chemicals is similar to nutrient uptake process. Due to their lipophilic and adsorptive nature the uptake of such chemicals occurs at a faster rate till the equilibrium with concentrations in the water is maintained (AUER et al. 1982; NAU-RITTER et al. 1982). The delayed effect of endosulfan, observed in the present experiment might be due to such concentration dependent accumulation of the pesticide with the alga. This, in turn, increased the concentration inside the cells causing lowering og LEC value with increae in age of the cultures.

Though a slight inhibition of pigment content of C. vulgaris was observed at 1 mg/L of dimethoate on the first day of observation, it was not so on all other subsequent occasions, and in stead, a significant increase in pigment biomass was found after 7 days similar to growth rates (Fig. 4A). The other higher concentrations, however, caused reduction of pigment content which was both dose and time dependent. The rates of inhibition at each
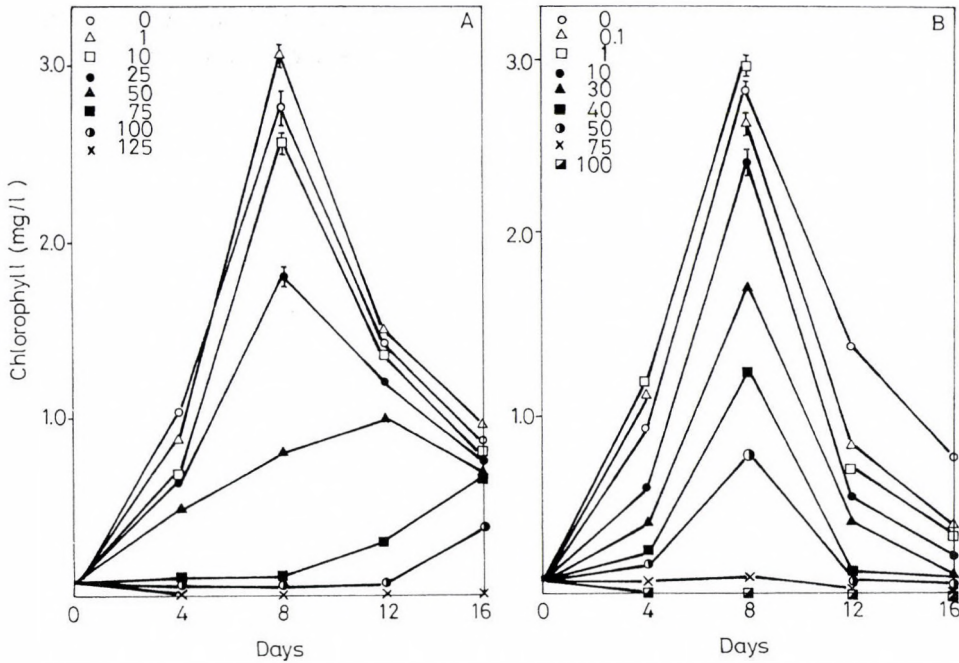
Fig. 4. Effect of (A) dimethoate and (B) endosulfan concentrations (mg/l) on the pigment biomass (mg/l) of C. vulgaris

tested concentration of the pesticide (exception being at 1 mg/L) were always found significant. The significant difference between the mean values of pigment biomass of any two concentrations in all the observations indicated that the toxicity effect of the pesticide varied remarkably at different concentrations. As in case of growth, the pigment biomass remained static at 100 mg/L of the chemical till the 12th day and a significant increase was recorded thereafter whereas at 125 mg/L practically there was no pigment till the end of incubation.

Unlike in case of dimethoate, a direct relationship between the endosulfan toxicity and incubation period of the culture was noted (Fig. 4B). The toxicity effect was, however, found to be directly related to the concentration of the chemical as in case of dimethoate. At 0.1 and 1.0 mg/l of the pesticide compared to the control a significant acceleration of pigment content was observed on the 4th day while on the 8th day it was so only at 1.0 mg/L. At 0.1 mg/L, on the other hand, pigment biomass was insignificantly lower than that in the control on the 8th day indicating the beginning of inhibitory action of the dose from this day. The pesticide was found effective in reducing the pigment content at doses 10 mg/L from the 4th day and at all the tested concentrations from the 12th day onwards. Comparing the

mean values of pigment biomass at each concentration always it was found that toxicity effects of the chemical at different dose are significantly different from one another irrespective of the age of the cultures. Like growth, a slight increase of pigment biomass at 75 mg/L of the pesticide was observed on the 8th day (compared to initial value) while decreasing on subsequent observations. The pigment content at 100 mg/L of the pesticide was nil.

The low values of the pigment biomass at the presence of different concentrations of the pesticides may be due to both inhibition of synthesis and augmentation of degradation processes. This may be due to the fact that the pesticide interferes with synthesis of photosynthetic pigments by inhibiting the formation of perphyrin and the biosynthetic steps in the carotenoid synthesis pathway which ultimately leads to chlorosis and accumulation of precursors (EDWIN 1977, MORELAND 1980). Such accumulation, in turn, makes unnecessary locking of cellular compound affecting further metabolic efficiency. In the present experiment a direct relation between growth and pigmentation was observed. The rate of inhibition of growth was more or less synchronous with that of pigment biomass but, however, the former was more than the latter at higher concentrations of both the pesticides. It indicates the fact that the pesticides not only affect the pigment biomass but also regulate their function. ANTUNES-MADEIRA et al. (1980) reported that pesticides interact with lipid bilayer of the chloroplast membrane and produce disordering effects by affecting lipid packing with increased motion of the lipid hydrocarbon chain of the phospholipids. This causes permanent physical and chemical changes in the chloroplast membrane. At higher concentrations pesticides cause imbalances in the function of thylakoids making polyhedral bodies incapable of performing their function (METHA and HAWXBY 1979). As a result the H_2O-DCIP (or FeCN) electron transfer of PS II is hampered (ANBUDURAI et al. 1981). As a result the rate of photosynthesis is reduced leading to impairing of cell division and finally reduction of rate of the growth of algae.

The O.D. values of cultures, subjected to the three higher concentrations of the pesticides have been represented in Table 1. The results showed that both the pesticides were detoxified at each of the tested concentrations by the alga. The rate of detoxification, however, varied with the doses and type of the pesticide. In case of dimethoate the growth rates were 25.0 ± 1.51%, 7.5 ± 0.0% and 3.78 ± 0.08% of the control at 75, 100 and 125 mg/L, respectively after the first inocultion while they were increased to

Table 1

Detoxification of the pesticides by Chlorella vulgaris on repeated removal and reinoculation procedure (mean OD of the cultures in triplicates)

Inoculations	Concentrations (mg/L)							
	Dimethoate				Endosulfan			
	0	75	100	125	0	20	30	40
First	0.132^a (0.003)	0.033^a (0.002)	0.010^a (0.0)	0.005^a (0.0)	0.134^a (0.005)	0.097^a (0.003)	0.052^a (0.002)	0.033^a (0.001)
Second	0.133^a (0.002)	0.090^b (0.005)	0.077^b (0.002)	0.054^b (0.002)	0.138^a (0.004)	0.111^b (0.002)	0.078^b (0.005)	0.060^b (0.002)
Third	0.128^a (0.002)	0.114^c (0.001)	0.104^c (0.003)	0.081^c (0.001)	0.124^a (0.001)	0.124^c (0.002)	0.107^c (0.004)	0.083^c (0.001)

1. The values in the parantheses are standard errors of triplicates.
2. The different letters indicate significant differences between the values (p = 0.05) of each column.

89.06 ± 0.78%, 81.25 ± 2.34% and 63.28 ± 0.78% at the respective doses after the third. Similarly in case of endosulfan the growth increased from the first towards the third inoculation at the rate of 72.38 ± 2.24%, 31.81 ± ± 1.49% and 24.64 ± 0.75% to 100 ± 1.61%, 86.29 ± 3.32% and 66.93 ± 0.89% of the control at 20, 30 and 40 mg/L, respectively. The difference between the O.D. values between any two inoculations at any concentration of the pesticides showed that there was significant increase in growth rate with increase in the number of inoculations. The results showed that dimethoate was more successfully detoxified than endosulfan by the test alga. This may be due to more rapid uptake of dimethoate from the medium by repeated removal and reinoculation of the alga as well as due to biodegradation of the chemical (MOHAPATRA et al. 1990).

Table 2 showed the growth of C. vulgaris in repsonse to the three selected concentrations of dimethoate at different cell density level. At LCD the O.D. and log cell numbers of each treated culture were always found to be lower than the control. The growth inhibition by the selected concentrations was significant even from the very first observation (2nd day) and the values at each concentration significantly differed from each other and the control. At 51 mg/L of the pesticide the test alga remained static till the 4th day though not so thereafter but complete death was recorded at 102.1 mg/L. At MCD the O.D. and cell count values at each concentration of the pesticide were significantly lower than that of the control and there were

Table 2

Effect of cell density of *Chlorella vulgaris* on the toxicity effects of dimethoate

Cell density ($x10^6$/mL)	Conc. mg/L	Days 2 A	2 B	4 A	4 B	6 A	6 B	8 A	8 B
0.35	0	0.20^{a} (0.00)	6.75^{a} (0.00)	0.035^{a} (0.002)	7.003^{a} (0.029)	0.063^{a} (0.001)	7.25^{a} (0.012	0.114^{a} (0.002)	7.507^{a} (0.011)
	10	0.010^{b} (0.00)	6.46^{b} (0.00)	0.020^{b} (0.00)	6.75^{b} (0.00)	0.050^{b} (0.00)	7.163^{b} (0.016)	0.103^{b} (0.004)	7.46^{b} (0.021)
	51	0.008^{c} (0.00)	6.35^{c} (0.00)	0.008^{c} (0.00)	6.35^{c} (0.00)	0.034^{c} (0.002)	6.98^{c} (0.039)	0.075^{c} (0.001)	7.327^{c} (0.015)
	102.1	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}
1.40	0	0.038^{a} (0.001)	7.03^{a} (0.024)	0.050^{a} (0.00)	7.150^{a} (0.00)	0.093^{a} (0.001)	7.42^{a} (0.012)	0.122^{a} (0.002)	7.523^{a} (0.008)
	10	0.035^{b} (0.001)	6.977^{b} (0.008)	0.047^{b} (0.002)	7.120^{b} (0.012)	0.08^{b} (0.00)	7.34^{b} (0.012)	0.115^{b} (0.00)	7.153^{b} (0.011)
	51	0.025^{c} (0.001)	6.863^{c} (0.016)	0.032^{c} (0.001)	6.943^{c} (0.029)	0.060^{c} (0.003)	7.237^{c} (0.008)	0.090^{c} (0.001)	7.403^{c} (0.011)
	102.1	0.005^{d}	6.15^{d} (0.00)	0.005^{d}	6.15^{d} (0.00)	0.005^{d}	6.15^{d} (0.00)	0.005^{d}	6.15^{d} (0.00)
2.45	0	0.048^{a} (0.001)	7.137^{a} (0.018)	0.092^{a} (0.001)	7.41^{a} (0.004)	0.113^{a} (0.002)	7.503^{a} (0.004)	0.145^{a} (0.003)	7.61^{a} (0.00)
	10	0.045^{a} (0.00)	7.10^{a} (0.00)	0.088^{a} (0.00)	7.39^{b} (0.00)	0.130^{a} (0.002)	7.457^{a} (0.016)	0.145^{a} (0.004)	7.597^{a} (0.008)
	51	0.034^{b} (0.002)	6.98^{b} (0.039)	0.069^{b} (0.001)	7.287^{b} (0.008)	0.078^{b} (0.002)	7.333^{b} (0.029)	0.115^{b} (0.003)	7.153^{b} (0.020)
	102.1	0.018^{c} (0.00)	6.70^{c} (0.00)	0.020^{c} (0.00)	6.70^{c} (0.00)	0.026^{c} (0.001)	6.863^{c} (0.016)	0.035^{c} (0.002)	7.01^{c} (0.024)

1. The values in the parentheses are standard errors of triplicates.
2. Columns A and B represent data on growth (OD) and log cell number/mL, respectively.
3. Different letters indicate significant differences between the values of each column ($p = 0.05$).

also significant differences between the mean values of the treated cultures. However, at this cell density level, unlike in case of LCD, growth was found from the very first observation at 51 mg/L of the pesticide, and there were continuous increases in the OD and mean cell counts with increase in the age of the culture. The toxicity effect of the pesticide was somewhat different at HCD. Though no change in the toxicity effect of the pesticide was observed with increase in the age of the culture irrespective of the

Table 3

Effect of cell density of Chlorella vulgaris on the toxicity effects of endosulfan

Cell density ($x10^6$/mL)	Conc. mg/L	Days 2 A		4 A		6 A		8 A	
		A	B	A	B	A	B	A	B
0.35	0	0.028^{a} (0.002)	6.883^{a} (0.037)	0.045^{a} (0.00)	7.120^{a} (0.010)	0.072^{a} (0.002)	7.300^{a} (0.010)	0.115^{a} (0.001)	7.517^{a} (0.003)
	1	0.015^{b} (0.00)	6.62^{b} (0.00)	0.025^{b} (0.00)	6.85^{b} (0.00)	0.063^{b} (0.001)	7.243^{b} (0.009)	0.080^{b} (0.00)	7.350^{b} (0.001)
	41.5	0.005^{c} (0.00)	6.15^{c} (0.00)	0.005^{c} (0.00)	6.15^{c} (0.00)	0.015^{c} (0.00)	6.45^{c} (0.00)	0.018^{c} (0.00)	6.70^{c} (0.0)
	78.6	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}
1.40	0	0.045^{a} (0.00)	7.093^{a} (0.007)	0.063^{a} (0.002)	7.25^{a} (0.010)	0.093^{a} (0.002)	7.42^{a} (0.010)	0.105^{a} (0.005)	7.473^{a} (0.003)
	1	0.035^{b} (0.00)	7.003^{b} (0.013)	0.053^{b} (0.001)	7.17^{b} (0.012)	0.085^{b} (0.00)	7.383^{b} (0.003)	0.120^{b} (0.003)	7.597^{b} (0.009)
	41.5	0.005^{c} (0.00)	6.15^{c} (0.00)	0.010^{c} (0.00)	6.15^{c} (0.00)	0.015^{c} (0.00)	6.62^{c} (0.00)	0.053^{c} (0.003)	7.178^{c} (0.028)
	78.6	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}
2.45	0	0.045^{b} (0.00)	7.083^{b} (0.017)	0.072^{b} (0.002)	7.29^{b} (0.03)	0.113^{b} (0.003)	7.503^{b} (0.013)	0.125^{b} (0.003)	7.543^{b} (0.012)
	1	0.055^{a} (0.003)	7.183^{a} (0.027)	0.090^{a} (0.00)	7.373^{a} (0.012)	0.137^{a} (0.004)	7.583^{a} (0.015)	0.155^{a} (0.008)	7.59^{a} (0.031)
	41.5	0.020^{c} (0.00)	6.75^{c} (0.00)	0.035^{c} (0.00)	6.99^{c} (0.00)	0.058^{c} (.003)	7.213^{c} (0.023)	0.077^{c} (0.001)	7.33^{c} (0.01)
	78.6	0.005^{d} (0.00)	6.45^{d} (0.00)	0.010^{d} (0.00)	6.45^{d} (0.00)	0.020^{d} (0.00)	6.45^{d} (0.00)	0.025^{d} (0.00)	6.81^{d} (0.00)

1. The values in the parentheses are standard errors of triplicates.
2. Columns A and B represent data on growth (OD) and log cell number/mL, respectively.
3. Different letters indicate significant differences between the values of each column ($p = 0.05$).

dose, it was nevertheless different from that observed in case of LCD and HCD. At 10 mg/L of the pesticide, inhibition of growth at HCD was observed in the 2nd, 4th and 6th day as in case of the other two cell density levels whereas on the last day of observation the growth at this concentration was similar to that in the control. However, unlike in case of the other two cell density levels the inhibitory action of 10 mg/L of the pesticide was insignificant at HCD though it was significant at other two higher concentrations.

Similarly in case of endosulfan, the three tested concentrations caused significant reduction of growth from the very first observation (2nd day) at LCD (Table 3). Substantial reduction in the growth rate was found in all concentrations tested and even at the lowest concentration (1 mg/L) throughout the incubation period though a comparative decrease in the inhibition of growth rate at this concentration was observed with increase in the age of the culture. Though the alga was found growing at 41.5 mg/L at this cell density (low cell density at the initial stage) level the growth rate was very slow compared to that in the control and there was complete death of the cells at 78.6 mg/L of the pesticide from the beginning. At MCD, however, 1 mg/L of the pesticide was found less toxic to the alga, though OD and mean cell count values at this concentration were significantly lower than that of the control at MCD in the first three observations, they were found to be significantly higher on the 8th day indicating the reversal of toxicity of the dose. Similarly, though the growth was very slow till the 6th day at 41.5 mg/L of the pesticides, thereafter, at this cell density level, it substantially increased. At 78.6 mg/L, no cell was found alive. At HCD, on the other hand, 1 mg/L of the pesticide was found growth stimulating throughout the incubation period. The rate of growth acceleration at this concentration and cell density level was significant which remained unchanged with increase in the age of the culture. At the other two higher concentrations, though growth was significantly inhibited, there was gradual increase in the rate of growth in response to age of the culture till the 8th day.

From the results it is clear that the initial cell density has significant influence in regulating the toxicity effect of the pesticides. In the present experiment the LECs and the static concentrations (SCs) of the pesticides for the alga were changed with change in the initial cell density. For example 10 mg/L of dimethoate was significantly inhibitory to the alga at LCD and MCD while it was not so at HCD though insignificant inhibition occurred at this concentration. Similarly 102.1 mg/L was cidal to the alga at LCD, static at MCD where as at this concentration growth was recorded at HCD. The low density of cells at initial level made the alga highly sensitive to all the concentrations of endosulfan and at 1 mg/L the growth inhibition rate was as high as 30% of the control after 8 days of incubation. On the other hand, the same concentration was significantly growth stimulatory after 6 days at MCD and from the beginning at HCD. At 78.6 mg/L complete death of the algal cells occurred at LCD and MCD while the growth

at this concentration was as high as 20% of the control after 8 days at HCD. We can conclude that the toxicity of a chemical is at least to a certain extent inversely proportional to the initial cell density level. Therefore even lower concentrations of each of the pesticide might become toxid to the microorganisms when their population levels are very low in the natural environment. This necessitates the importance of studying the existing population level of microorganisms while evaluating the toxicity of water borne pesticides. The effects of algicides (FITZGERALD 1975) and pesticides (MOHAPATRA et al. 1990) have been reported to be dependent on the initial cell density levels from which the cells start showing their responses. Subsequently increased tolerance at high cell density level may be due to high packed cell volume and/or decreased pesticide to organism ratio.

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EFFECTS OF LIGHT ON GROWTH, GLUCOSE UPTAKE AND SOME METABOLIC PROCESSES IN COLORLESS CHLORELLA KESSLERI MUTANT CELLS

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The effect of light on growth, glucose uptake and fatty acid composition of total and individual lipid classes in colorless Chlorella kessleri mutant cell has been studied. White light was inhibitory to the growth and glucose uptake and this inhibition was concomitant with the time of exposure of the cells to light. Light had a slight inhibition effect on dark respiration. The effect of light on fatty acid composition of total lipids and the individual lipid classes isolated from mutant cells of Chlorella kessleri was studied using gas liquid chromatography. The results indicated that light stimulated the monounsaturated fatty acid synthesis while the unsaturated fatty acid C18:3 is enhanced in the dark grown cells. The relationship between light-stimulation of fatty acids synthesis also was discussed.

Introduction

The colorless mutant Chlorella kessleri is a unicellular alga which lack chlorophyll, can utilize organic carbon source in the dark or in the light. The inhibition of glucose uptake by light has been found in Chlorella pyrenoidosa (ANDERSAG and PIRSON 1976), Prototheca zopfii (EPEL and KRAUSS 1966), Euglena gracilis (NICOLAS et al. 1980) and Chlorella vulgaris (KAMIYA 1985). This inhibition is a low light reaction and also observed in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea. Thus, it is not related to photosynthesis but little else is known about the process of light-induced inhibition of glucose uptake in algae. The main effect of light on

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Abbreviations: DGDG = digalactosyl diacylglycerol; MGDG = monogalactosyl diacylglycerol; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PG = phosphatidylglycerol; PI = phosphatidylinositol; SQDG = sulfoquinovosyl diacylglycerol; UI = unsaturation index; Un/S = polyne index B; 12:0 = lauric acid; 14:0 = miristic acid; 16:0 = palmitic acid; 16:1 = = hexadecenoic acid; 16:2 = hexadecadienoic acid; 18:0 = stearic acid; 18:1 = oleic acid; 18:3 = linolenic acid.

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dark-grown Euglena cells is to induce chloroplast development (NIGON and HEIZMANN 1978). Other effects of light include inhibition of the activity of glyoxylic enzymes (COOK and CARVER 1966; DAVIS and MERRET 1974), decrease of the growth rate of mutants (COOK and KAISER 1973).

In colorless mutant (KAMIYA and MIYACHI 1974) as well as wild-type cells (MIYACHI et al. 1980) of Chlorella vulgaris 11 h, dark CO_2 fixation, which is initiated by phosphoenolpyruvate carboxylase, as well as endogenous respiration, is suppressed during starvation in phosphate medium in the dark. Recently, KAMIYA (1991) indicated that addition of CA^{2+} enhanced glucose uptake in a colorless mutant of Chlorella kessleri illuminated by blue light. NICOLAS (1965) studied the fatty acid composition of wild-type Chlorella vulgaris grown on a purely inorganic medium in the light and on organic medium both in the light and in the dark. He found that cells grown in the light on an inorganic medium contain more a-linolenic acid than do those grown on an organic medium. He also found that light had little effect on the fatty acid composition of cells grown on an organic medium. NICHOLS and APPLEBY (1969) indicated that the isolated lipids of Chlorella are similar to those occurring in the leaves, since polyenoic acids of the C16 and C18 series are predominantly localized in the galactosyl diglycerides (MGDG). Recently, PICAUD et al. (1991) indicated that fatty acid biosynthesis is stimulated by light in Chlamydomonas reinhardtii, they concluded that the mechanism of activation remains poorly understood. The present experiments were undertaken to see whether light has an influence on growth, glucose uptake and fatty acid biosynthesis from colorless Chlorella kessleri mutant cells.

Material and Methods

Organism and culture conditions

The colorless Chlorella kessleri Fott et Novaková, no. 9.80 (Sammlung von Algenkulturen, Pflanzenphysiologisches Institut der Universität Göttingen, Germany) was used in this investigation. The alga was grown in a solution of iorganic salts (24 mM potassium nitrate as the source of nitrogen) plus 55.56 mM glucose, at 27 ^{0}C (KAMIYA 1985). The culture flasks were shaken horizontally with circular motion at 100 cycles/min either in the dark or in the light (40 $\mu E\ m^{-2}\ S^{-1}$).

Measurement of growth

The growth of the cultures was monitored by counting the cells by means of 0.1 mm deep haemocytometer slide. The mean of 16 squares was taken for each measurement.

Measurement of glucose

The glucose content of the medium was determined by hexokinase method (Gluco-quanto; Boehringer Mannheim GmbH, Mannheim).

Measurement of oxygen uptake

Oxygen uptake was determined with a Clark-type electrode at 27 °C in 3 ml sample.

Measurement of protein

The protein content of the cells was estimated by the method described by LOWRY et al. (1951).

Analysis of lipids

Lipids were extracted from 10 ml aliquots of culture by the method of BLIGH and DYER (1959). The analysis was carried out by the method of SATO and MURATA (1988). The lipid classes were separated by thin-layer chromatography on precoated silica-gel plates (5721-Merck, Darmstadt, Germany) with a mixture of chloroform/methanol/acetic acid/water (85:15:10:3, by vol.) as the mobile phase (NICHOLS et al. 1965). After development, the plates were dried in a stream of CO_2 and the lipid classes were identified using 8-anilinoaphthalin-sulphate (ANS) fluorescence elution and DGDG, MGDG, PC, PE, PI, PG and SQDG (Seradary Research Lab.) as standards. The separated lipids were taken into ampoules, containing 5% (W/V) HCl in dry methanol at 85 °C for 2.5 h under N_2. The resultant methyl esters of the fatty acids were extracted from esterification mixture after dilution with an equal volume of water by n-hexane.

Gas chromatography

Methyl esters were analyzed on gas liquid chromatographic system (Hewlett—Packard 5890 sieries II) equipped with a capillary column coated with SP 2330 of 0.25 μm thickness (0.25 mm i.d.x30 mm, CPS-Li Quardex, New Haven, CT. USA). High purity nitrogen was applied at a flow rate 230 KPa, hydrogen 100 KPa and oxygen 280 KPa. The dual column system was programmed from 160 °C to 200 °C to give partial separation of C18:3 at the rate 2.5 °C min^{-1}. The detector temperature and injector temperature was 220 °C. Identification of the peaks was made using linoleonic standard and by plotting log. relative elution temperature versus the number of carbon atoms (SCHMIDT and WYNNE 1967). To calculate the percentage composition using Hewlett—Packard 3396 A integrator, all peaks emerging between lauric (12:0) and linolenic (18:3) were included in calculations.

Results and Discussion

When Chlorella kessleri mutant cells was transferred from an aga-agar slant to a glucose medium and shaken in the dark, a linear increase in growth as followed by the counting of the cells was observed (Fig. 1). In culture illuminated by cool-white fluorescent lamps, it was found that light was indeed inhibitory to growth in all three periods tested. EPEL and KRAUSS
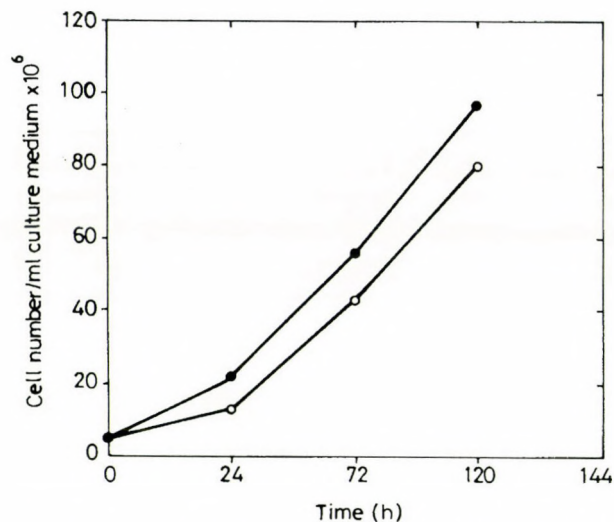
Fig. 1. Effect of light on growth curve of colorless mutant Chlorella kessleri. The growth was followed by the increase in cell number counting. Light grown cells (o) and dark grown cells (●)
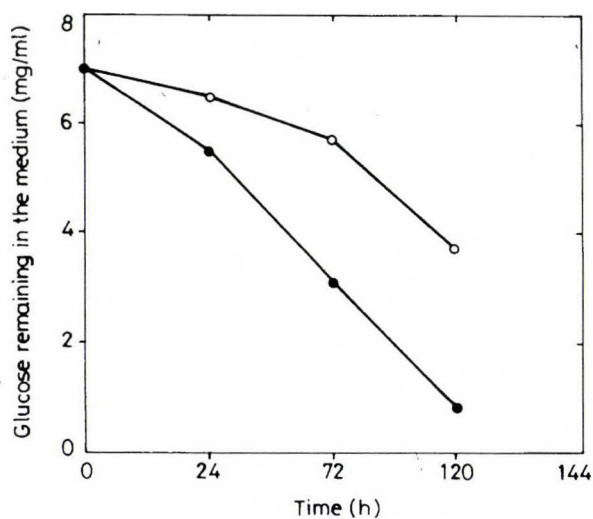
Fig. 2. Effect of light on glucose content in the medium in colorless mutant cells of Chlorella kessleri. Light grown (o) and dark grown cells (●). The initial concentration of glucose was 7 mg/ml suspension. Other conditions in Material and Methods

(1966) found that white light inhibited the growth of Prototheca zopfii and this inhibition increased by increasing light intensity. KAMIYA (1985) observed that colorless mutant Chlorella vulgaris growth inhibited in the

Table 1

Effects of light on dark respiration ($\mu mol\ O_2 \cdot mg\ protein^{-1} \cdot h^{-1}$) and protein content ($mg \cdot ml^{-1}$) in mutant cells of Chlorella kessleri

Age (h)	Dark		Light	
	Protein	Respiration	Protein	Respiration
24	0.006 (100)	136 (100)	0.005 (83)	163 (120)
72	0.045 (100)	51 (100)	0.038 (84)	54.5 (89)
120	0.057 (100)	36 (100)	0.051 (89)	32 (89)

light. He reported also that blue light strongly inhibited growth but red light only slightly affected it. To follow up the glucose uptake by the cells grown in dark or in the light, we measured the glucose content remaining in the medium at different time intervals (Fig. 2). It was evident that, light strongly inhibited glucose uptake and this inhibition increased by increasing the exposure of the cells to light. Glucose uptake was inhibited by 15%, 46% and 78% after 24, 72 and 120 hours, respectively. Glucose uptake by Euglena was inhibited in the presence of light and the extent of inhibition was a function of light intensity (NICOLAS et al. 1980). KAMIYA (1985, 1991) and KAMIYA and KOWALLIK (1987) indicated that white and blue light induced inhibition of glucose uptake in Chlorella. Protein synthesis was also inhibited by the light in C. kessleri mutant cells (Table 1). The extent of inhibition decreased with increase in age of the culture. On the other hand, light had a slight inhibitory effect (11%) on dark respiration throughout the incubation period.

Lipid of Chlorella mutant cells

We have studied the effect of light on fatty acid composition of total lipids and the individual lipid classes isolated from C. kessleri mutant cells. Values in Table 2 represent the fatty acid composition after 24, 72 and 120 hours growth either in the dark or in the light. The analysis of fatty acid shows that the predominant fatty acids in C. kessleri mutant cells were C16:0, C18:1cis and C18:1trans. In C. kessleri wild-type, the predominant fatty acids of total lipids were C16:0, C16:2, C18:2 and C18:3 (EL-SHEEKH and RADY 1992). We also reported that C. kessleri forms C16:3 and C18:2 which were not identified in mutant cells of C. kessleri either in total lipids or in the individual lipid classes (Table 2). The fatty acid

Table 2

Fatty acid composition of the total lipids and the individual lipid classes isolated from the colorless mutant cells of *Chlorella kessleri* grown either in the dark or in the light

Lipid	Treat-ment	Age (h)	Fatty acids (mol%)										Sat	Mono	St/un
			12:0	14:0	16:0	16:1	16:2	18.0	18:1t	18:1c	18:2	18:3			
Total lipid	Dark	24	4.14	5.72	16.8	7.44	6.42	5.29	12.7	33.4	—	8.12	41.95	53.7	2.13
		72	3.64	2.30	19.1	4.94	4.21	2.01	7.80	46.4	—	8.98	26.99	59.2	2.7
		120	2.23	1.47	19.68	3.36	2.81	2.01	8.18	51.67	—	8.58	25.4	63.2	2.94
	Light	24	7.04	4.67	22.02	6.17	3.59	4.41	13.52	31.10	—	7.47	38.1	5.8	1.62
		72	5.28	2.49	19.16	3.68	2.80	1.87	9.10	47.51	—	8.06	28.8	60.3	2.47
		120	2.34	1.82	18.14	3.55	1.92	1.55	8.80	53.54	—	8.31	23.85	65.9	3.2
PC	Dark	24	—	1.89	14.85	5.48	4.60	0.47	8.02	49.02	—	15.67	17.2	62.5	4.81
		72	—	0.86	1.97	4.8	3.0	1.3	6.90	60.60	—	11.89	13.1	71.6	6.63
		120	—	1.26	12.40	4.50	3.00	1.45	5.67	61.66	—	9.86	15.11	71.8	5.60
	Light	24	—	4.49	20.4	1.74	3.12	2.28	12.76	36.50	—	10.06	26.8	60.0	2.66
		72	—	1.11	14.54	3.39	1.35	1.73	10.0	59.0	—	9.08	17.4	72.4	4.75
		120	—	—	9.52	5.59	2.29	—	6.67	65.1	—	10.82	9.50	77.5	9.50
PE	Dark	24	—	4.64	19.77	—	5.66	1.66	—	58.0	—	10.33	26.1	58.0	2.80
		72	—	1.09	31.83	—	2.69	0.57	3.70	51.22	—	8.91	33.5	54.9	2.0
		120	—	0.22	32.85	—	1.59	0.44	2.13	55.38	—	7.39	33.5	57.5	2.0
	Light	24	—	—	8.0	—	0.92	7.98	25.37	45.81	—	11.91	16.0	7.12	5.3
		72	—	—	28.94	—	1.69	0.41	3.30	57.48	—	8.18	29.3	60.8	2.41
		120	—	0.70	32.18	—	—	8.53	1.45	57.35	—	7.79	33.4	28.8	2.0

Sat = saturated fatty acids; Mono = monounsaturated fatty acids; St/un = ratio of saturation: unsaturation

Table 3

Fatty acid composition of the individual lipid classes isolated from the colorless mutant cell of *Chlorella kessleri* grown either in the dark or in the light

Lipid	Treat-ment	Age (h)	Fatty acids (mol%)									Sat	Mono	St/un
			14:0	16:0	16:1	16:2	18:0	18.1t	18:1c	18:2	18:3			
MGDG	Dark	24	2.10	5.34	8.70	31.25	1.80	9.61	34.39	—	6.33	9.74	52.70	9.2
		72	1.66	4.32	5.74	21.02	1.13	8.27	46.09	—	11.75	7.11	60.1	13.1
		120	1.11	4.27	5.01	20.69	—	4.39	51.02	—	15.70	5.1	6.6	17.5
	Light	24	1.71	11.62	10.64	16.07	2.52	13.72	35.56	—	8.16	15.85	50.32	5.3
		72	—	4.29	7.83	19.25	0.81	8.72	44.94	—	14.18	5.1	61.5	18.6
		120	—	7.44	7.47	14.74	—	8.05	49.29	—	13.02	7.4	64.8	12.4
DGDG	Dark	24	3.07	37.42	9.49	3.60	0.56	19.41	11.67	9.63	5.85	41.0	4.6	1.4
		72	2.75	53.70	8.00	2.21	1.10	13.26	13.89	2.21	3.31	57.1	35.1	0.75
		120	1.46	62.04	7.45	3.82	1.64	7.58	15.06	—	0.92	65.1	30.1	0.536
	Light	24	—	56.43	—	—	5.3	21.18	17.10	—	—	61.7	38.3	0.62
		72	0.33	47.92	—	—	3.44	16.93	29.08	—	2.50	51.7	46.0	0.93
		120	1.38	42.18	—	—	1.66	12.31	34.43	3.59	4.23	45.5	46.7	1.20

Sat = saturated fatty acids; Mono = monounsaturated fatty acids; St/un = ratio of saturation: unsaturation

12:0 was present in C. kessleri mutant cells in the total lipids while it was not identified in the lipid classes. The fatty acids of the total lipids contain also higher amount of saturated fatty acids (C16:0) and monosaturated fatty acids (18:1 cis) which they have a closed relation with light growth. It can be assumed that light stimulates fatty acid synthesis via stimulate production of ATP, which acts as a source of adenylated energy and activation of several enzymes, e.g. acetyl-CoA carboxylase, this enzyme regulate synthesis pathway of fatty acids (STUMPF 1970; ESTWELL and STUMPF 1983). Table 2 shows also a large differences in the fatty acid composition of PC and PE. In PC the most abundant fatty acids are (C18:1cis) and (C18:3) they comprise about 49% and 15% of the total fatty acids, respectively. The fatty acid 18:1cis increased with the increase in age of the culture maintained in the light, while 18:3 decreased in the same conditions. Our data showed also that, unsaturation degree was more higher in the exponential growth phase of the dark grown alga in contrast to the light grown alga which exhibited an increase in the stationary phase of growth (120 hours). Concerning PE, the unsaturation degree was more higher in the light grown cells than in the dark grown cells. This is an indication for high production of ATP which subsequently increase activation of acetyl-CoA carboxylase, and the last stimulate desaturation of fatty acids. The fatty acid 18:3 is more characteristic for heterotrophically grown than for heterotrophically grown algae and is concentrated in non-photosynthetic membranes of the cell (ERWIN 1973). Our results showed that, a relative amount of 18:3 fatty acid is enhanced in dark grown cells and it concentrated in PE and PC. These results are in agreements with the results obtained by EICHENBERGER (1976). The results in Table 3 show a marked differences in glycolipids fatty acids especially MGDG and DGDG. In MGDG it can be seen that C16:3 and 18:1cis are the main components of the fatty acids. C16:2 was decreased in the dark grown cells but it increased in the light grown cells till 72 hours and then it decreased while C18:1cis increased in both the dark and light grown cells. DGDG lipid class is characterized by large amount of C16:0 and 18:1 trans and cis, they comprise about 37%, 19.4% and 12% of the total fatty acids, respectively. DGDG is also characterized by increasing the unsaturation degree in the light grown cells. It is noteworthy that the galactosyl diglycerides contain a higher proportion of polyunsaturated fatty acids than other lipids. Monogalactosyl diglyceride rather more of these acids than diagalactosyl diglyceride (ALLEN et al. 1964). These differences in fatty acid composition between MGDG and DGDG would seem to argue against

the hypothesis that MGDG is converted to digalactosyl by direct galactosylation (NICHOLS 1963), so the fatty acids synthesis are closely connected with the photosynthesis. From the above discussed results, it can be concluded that light interfers with growth and glucose consumption in colorless mutant cells of Chlorella kessleri, and this may be caused by regulation of glucose metabolism. Light also, interfers with fatty acid distribution among the cells. The synthesis of polyunsaturated fatty acids are decreased by light with a componsatory increase in monosaturated fatty acids.

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STUDIES ON RARE AIRBORNE FUNGAL SPORES AND CONIDIA IN HUNGARY

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During our survey from February to November 1990 we were able to identify 29 fungal taxa. From these, 18 were Ascomycetes, 9 Deuteromycetes and 2 Basidiomycetes. We noticed an especially high occurrence of Cladosporium on following days:

30th September	50 900 spores/m^3
11th July	378 000 spores/m^3
16th June	15 652 spores/m^3

The highest number of fungal particles present in the air was found on the 30th September with 54 000 units. The particles of Cladosporiums are included in the above number; when the Cladosporiums are excluded, the highest count was obtained on the 10th July with 6500 particles. The 2nd May brought the highest number of fungal taxa; 12 taxa were present that day. We found no special seasonality, however, the presence of fungal spores in the air clearly showed a noticeable increase in the second half of the vegetational period (June—October). Besides the usually common taxa we found several spores and conidia which were rather seldom occurring in our air samples. These are the following: Ascomycetes: Chaetomium spp., Hypoxylon sp., Quaternaria sp., Xylaria sp., Didimosphaeria sp., Aglaspora profusa, Heptameria uncinata, Leptospora sp., Paraphaeosphaeria michotii, Phaeosphaeria spp., Pleuroceras cryptoderis, Rebentischia unicaudata, Sporormiella sp., Ophiobolus acuminatus, O. anguilides, O. fruticum, Cucurbitaria spp., Pleospora spp. Basidiomycetes: Urocystis sp. Deuteromycetes: Asterosporium asterospermum, Curvularia sp., Diplocladiella scalaroides, Drechslera spp.

Introduction

There are nearly 100 000 fungal species known at present on the earth (HAWKSWORTH 1990). Our interest is focussed on those among them which are airborne (that is about 70% of all known fungi) and produce allergenic spores and/or conidia abundantly. It is well-known that microfungal spores play an important role in the allergies of sporal origin (GREGORY 1973; GRAVESEN 1979). Our data is the first ever systematically collected in Hungary.

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Our systematic analysis of the aerospores started in 1989. Some of our results concerning the allergenic spores and conidia abundant in Hungary are already published (JÁRAI-KOMLÓDI 1991). The present paper additionally deals with some of the fungal taxa which we identified during our analysis and which may be said to be rare in our air samples and are never found in any significant quantity. Also, they are seldom mentioned in the aerobiological literature.

Method

We continuously followed the daily fungal and conidial contents of the air with the help of the Burkard seven day volumetric spore trap from February to November. Permanent preparata were made from the contents of the trap and were subsequently analysed. The results were expressed as number of spores respectively conidia per cubic metre. The analysis was based on sporal respectively conidial morphology, that is form, size and colour. Data collected daily was fed into a computer and is beeing treated by a "quattro pro" type softwear.

Results and Discussion

Even though spores and conidia are generally very variable in their form, size, colour and sculpture, their identification can be rather difficult (ALLITT 1978). At times it might be difficult to decide if one is dealing with the spores or with the conidium of the anamorph genus (e.g. Cucurbitaria-Camarosporium).

The most serious problem encountered in the process of identification of the trapped airborne spores/conidia is that neither their host(s) nor the fruit body and stroma of the fungus, nor the morphological relationship between stroma and the fruit body are known, without which their identification can be very difficult.

In our experience in Hungary with the exception of the well-known and easily identifiable to the genus level conidia of Deuteromycetes like Cladosporium, Alternaria, Epicoccum, Stemphylium, Torula, the majority of the remaining spores and conidia belong to the Ascomycetes. Although the identification of the latter is not easy, it seems appropriate, and due to their proven allergenic properties (ALLITT 1986; FRANKLAND-GREGORY 1973), important, to group the spores and conidia we identified in our survey, morphologically.

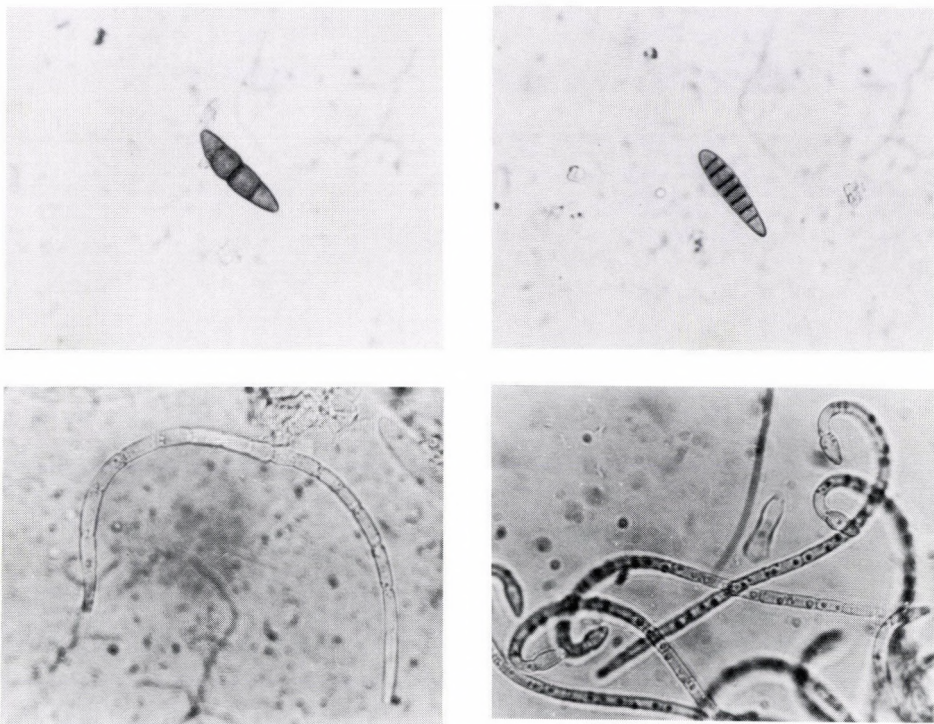



Plate I. Ascospores Ascomycetes

Fig. 1—2. Leptosphaeria typ. 27-28.5 x 7.5-8 µm. — Fig. 3. Ophiobolus compressus 150 x 2.5 µm. — Fig. 4. O. anguillides 120 x 3.5 µm

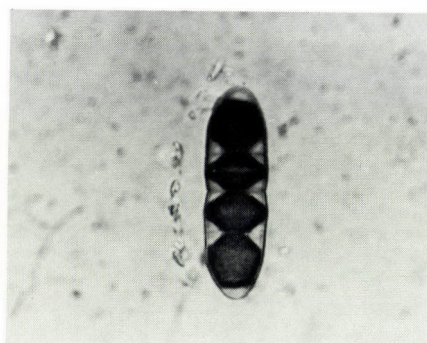



Plate II. Ascospores Ascomycetes

Fig. 5. Pleospora typ. 31.7 x 15 µm. — Fig. 6. Aglaospora profusa 52.5 x 15.3 µm. — Fig. 7. Cucurbitaria sp. 21.8 x 9.8 µm. — Fig. 8. Heptameria uncinata 40 x 5.6 µm. — Fig. 9. Hypoxylon sp. 12.3 x 7 µm

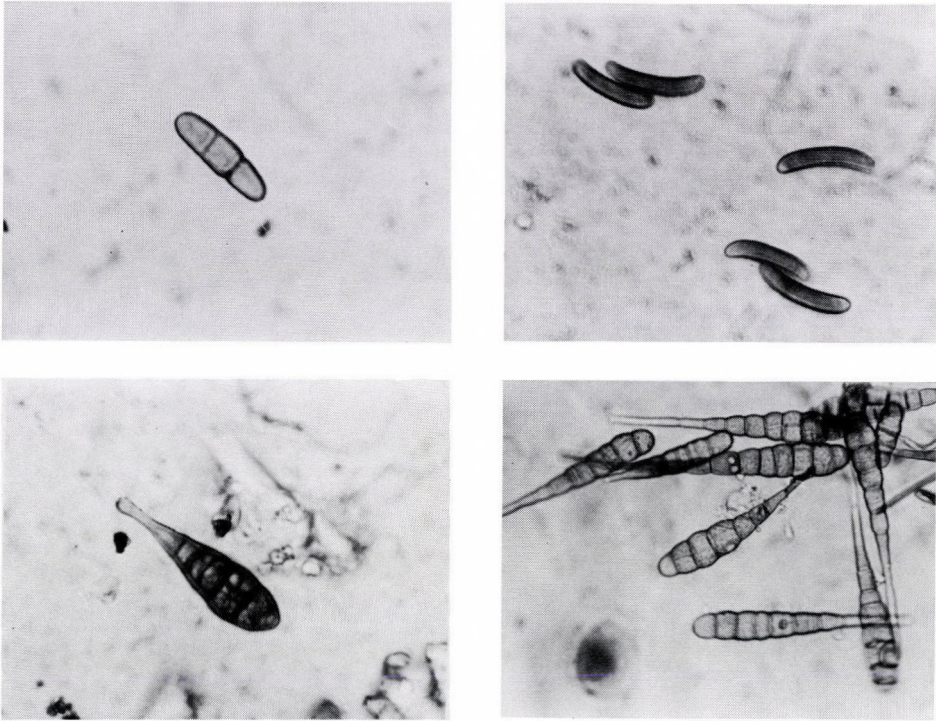



Plate III. Conidia of Deuteromycetes

Fig. 10. Paraphaeosphaeria michotii 17 x 4.8 µm. — Fig. 11. Quaternaria sp. 24 x 4.8 µm. — Figs 12—13. Alternaria sp. 40-56 x 9-12 µm

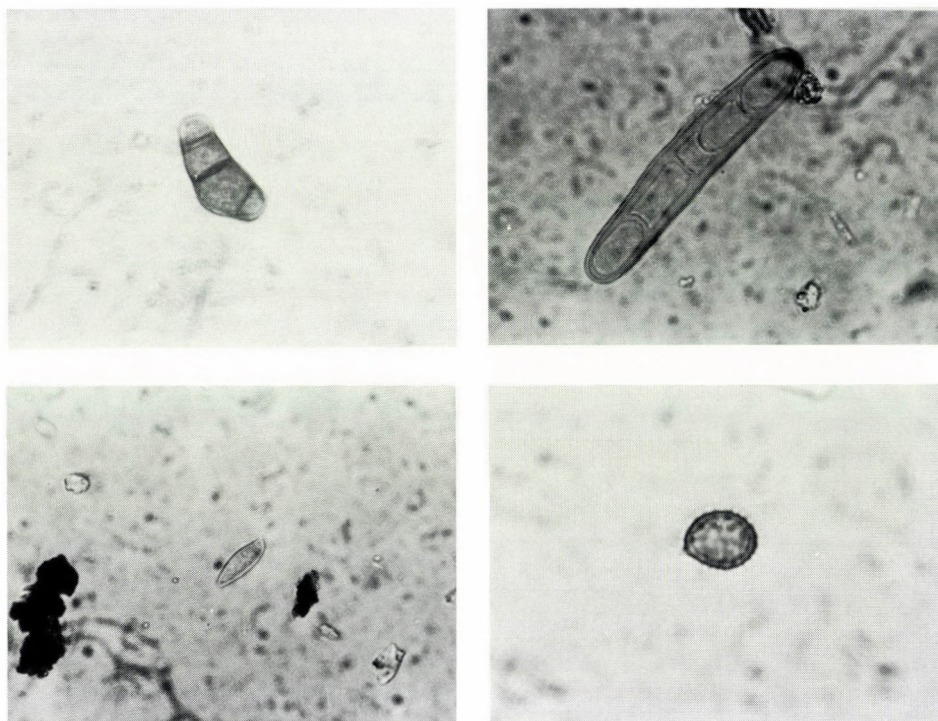
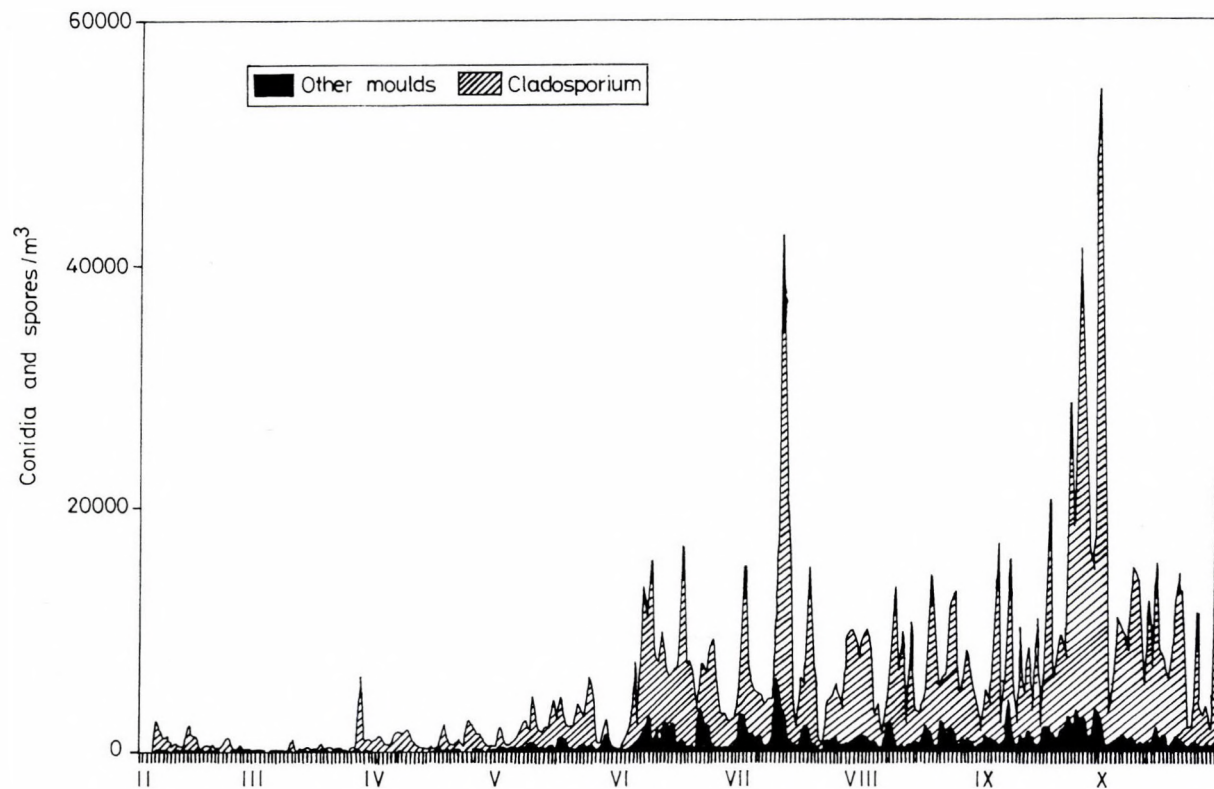



Plate IV. Conidia of Deuteromycetes and Basidiospores of Basidiomycetes

Fig. 14. Curvularia sp. 44 x 15.6 µm. — Fig. 15. Drechslera spp. 49 x 18 µm. — Fig. 16. Boletus typ. 15.3 x 5.7 µm. — Fig. 17. Lactarius typ. 9.3 x 7.5 µm

<u>Fig. 18.</u> Occurrence of aero-fungal particles in Hungary

Taxonomy and morphology of spores and conidia of rare fungi

Ascomycetes

A-1. Monocellate spores

a) Chaetomium-type

The spores are lemon-shaped, brown to blackish-brown, with one or two, more or less visible pore/s at the apex/apices and are 5-13 µm long, e.g. Chaetomium.

b) Allantoid-type

The spores are sausage-shaped and are either brown and 10-25 µm long, sometimes even longer, or colourless and generally much shorter, 5-15 µm long, e.g. Quaternaria.

c) Xylaria-type

Dark, either stubby or longish, spindle-shaped spores, frequently with unequal sides and a longitudinal germ slit. They are generally 10-25 µm long, but sometimes 8-35 µm, e.g. Hypoxylon.

All three types are easily distinguishable form each other.

A-2. Bicellate spore (rare ones). Didymosporae (Sacc.)

The spores are generally brownish, with one transvers septum. From the spores of the not so numerous genera belonging into this group, we found 8 spores of a Didymosphaeria species. The spores presumably originated from the same ascus, travelled in the air congregated and separated only during the process of preparation.

The spores of Delitschia are brownish-black, with a germ slit on at least one of the cells.

A-m. Tri- or multicellate spores

They usually are treated together as a group, even though at least three types are easily distinguishable: Phragmosporae, Scolecosporae, Dictyosporae according to P. A. SACCARDO (1882—1931).

a) Leptosphaeria-type (Table I/Figs 1—2; Table II/Fig. 7; Table III/ Fig. 2).

The name of the type is derived from the Leptosphaeria genus sensu lato, the genus most commonly found by us in the air. The former single genus was split taxonomically into several genera earlier in this century. Their identification cannot always be unequivocally done on the basis of spore morphology alone. This group comprises among others Leptosphaeria s. str.,

Phaeosphaeria, Paraphaeosphaeria, Rebentischia, Heptameria, which we found several times in air samples. Naturally, some other genera, with similar spores not yet found by us, belong as well into this type (Melanomma, Trematosphaeria etc.). Various spore forms are found here from the spindle-shaped through the stout and to the nearly cylindrical ones. Their common properties are the brownish colour, one or more swollen cells and several transversal septa. Their sizes vary as well: 15-55 µm x 3.5-25 µm.

b) Ophiobolus-type (Saccardo's Scolecosporae) (Plate I/Figs 3—4).
Like the Leptosphaeria genus, the Ophiobolus genus lato is also divided into several genera today. The spores are mainly brown, filiform and, generally articulated by 5-25 transvers septa. Those found by us measure about 80-150 µm x 2.5-4 µm.

c) Pleospora-type (Dictyosporae according to Saccardo) (Plate I/Fig. 5; Plate II/Fig. 6).
This type of spores is characterized by the stout, ellipsoidal (or slightly flattened) form, brown colour, and is multicellate having several longitudinal as well as transversal septa. The identification of the spores of this group might need some expertise. Common representants are Pleospora itself and the genus Cucurbitaria. Their differentation is only possible in the case of very characteristic spore forms.

The Pleospora develops mostly on dead stalks of herbaceous plants, while Cucurbitaria is found on dead branches of trees and shrubs. The conidial form (anamorphs) of the most common Pleospora species (P. herbarum /Pers./ Rabenh.) is Stemphylium botryosum Westend. Due to the extensive cultivation of Robinia pseudo-acacia L. in Hungary, the most common Cucurbitaria species is C. elongata (Fr.) Grev., its anamorph is Camarosporium sp. the conidium of which can be mistaken for spores of certain Pleospora or other Cucurbitaria species.

Deuteromycetes

As it has been observed in several investigations, the overwhelming majority of the allergenic viable microfungal species belong in this group (LARSEN-GRAVESEN 1991). After exclusion of the most frequent genera in Hungary (Cladosporium, Alternaria, Epicoccum, Torula, Stemphylium), following taxa have been (easily) identified from the rarer ones:

1. Asterosporium asterospermum (Pers. ex Gray/Hughes)
This is a brown, multicellate, four-branched conidium. The branches lie

approximately at 90 degrees from each other. They are conical, stout, 16-18 um wide at their bases. The tips of the branches are about 45-50 μm from each other.

2. Diplocladiella scalaroides Arn.

The conidium is brown, octocellate with three stout branches. One of the branches is cut off from the conidium at the point of the attachment to the conidiophore, the other two have transvers septa and are pointed. The cell lying at the far end is faintly coloured, ending in a very fine point.

3. Cercospora spp.

The conidium is wider at one end, slightly cut and tapering, with variable numbers of transvers septa (3-20), often very long, slender, brownish or hyalin, 40-150 μm x 3-5 μm.

4. Erysiphaceae spp. (anamorphs)

The conidia of the most common species of the Erysiphaceae are arranged is short chains. After disintegration of the chain, the conidia are monocellate, barrel-shaped and light yellowish or hyalin.

5. Drechslera spp.

The conidia are very variable in size, thick-walled, cylindrical in shape, obclavate or fusoid spindle-shaped, brown, with mostly 3-9 septa (distoseptate) and measure 40-250 μm x 14-22 μm. Most species of these fungi live parasitically on Graminea species, often on cereales.

Basidiomycetes

We found a few Puccinia teleutospores and aecidiospores from the Uredinales and a couple of Urocystis spores from the Ustilaginales. Further we found the very sporadically seen longish spores of Boletus (Plate IV/Fig. 3), the netlike sculptured spores of Lactarius (Plate IV/Fig. 4) from the Hymenomycetes, and finally a few spores of Myxomycetes. However, all these spores taken together were less numerous than e.g. the conidia of the genus Torula. As reported in the literature, the presence of these spores can in certain environmental conditions, be doubtless enhanced.

Occurrence and habits of the areospore producing fungi in Hungary

I. Ascomycetes

Aglaospora profusa (Fr.) de Not

Chaetomium spp.

Cucurbitaria spp.
Delitschia sp.
Didymosphaeria sp.
Heptameria uncinata (Niessl) Rehm
Hypoxylon sp.
Lasiosphaeria sp.
Ophiobolus acuminatus (Sow.) Duby
Ophiobolus anguillides (Cooke) Sacc.
Ophiobolus fruticum (Rob. ex Desm) Sacc.
Ophiobolus spp.
Paraphaeosphaeria michotii (Westend.) O. Eriks.
Phaosphaeria vagans (Niessl) Leuchtm.
Phaeosphaeria spp.
Pleospora spp.
Pleuorceras cryptoderis (Lév.) v. Höhn.
Quaternaria sp.
Rebentischia unicaudata (Berk. et Br.) Sacc.
Sporormiella sp.
Xylaria sp.

II. Deuteromycetes

Alternaria sp.
Asterosporium asterospermum (Pers. ex Gray) Hughes
Cercospora sp.
Cladosporium spp.
Curvularia sp.
Diplocladiella scalaroides Arn.
Drechslera spp.
Epicoccum purpurascens Ehrenbx ex Schlecht.
Erysiphaceae spp.
Stemphylium sp.

III. Basidiomycetes

Boletus sp.
Lactarius sp.
Puccinia sp.
Urocystis sp.

1. AGLAOSPORA PROFUSA

It is a characteristic, common, saprophytic fungus of Robinia pseudo-acacia

(intensively cultivated in Hungary), found mostly on dead branches fallen to the ground (Plate II/Fig. 2).

2. CHAETOMIUM SPP.

They are generally cellulose destroying saprophytic fungi. The spores are brown and have 1-2 germ pores. The hair-like appendages of the fruit bodies often form a characteristic crest-like group.

3. CUCURBITARIA SPP.

Live mainly on dead branches of woody plants forming conidia which grow in black, spherical groups from under the dead bark of the branches. The spores are brown, having at least one longitudinal and several transversal septa. Their distinction from the spores of Pleospora is only possible up to a certain degree. As the conidia appear on dying branches, the asco form is also rightly considered to be a parasite (Plate II/Fig. 3).

4. DELITSCHIA SP.

As it is a coprophylic fungus, its occurrence in the air is somewhat surprising.

5. DIDYMOSPHAERIA SP.

There are saprophytic as well as parasitic species in this genus. Eight spores of an ascus observed by us probably travelled, due to a sticky, mucous coating, congregated in the air and were caught as a group on the ribbon of the sampling apparatus.

6. HEPTAMERIA UNCINATA

A saprophytic fungus with characteristic spores living mostly on dead stalks of tall herbaceous plants, mainly on Artemisia vulgari and on Arctium and Cirsium species (Plate II/Fig. 4).

7. HYPOXYLON SP.

The perithecia push their spores out onto the nearly spherical or pillow-like surface of the stroma. The spores are spindle-like, deep brown to brownish-black in colour, sometimes with longish germ slit similar in this respect to Xylaria species. The spores of the two genera are mostly only distinguishable by their sizes. The fungus lives on dead twings of trees

8. LASIOSPHAERIA SP.

These species are presumably saprophytic, the spores of most species are characteristically nearly cylindrical long, hyalin or brownish in colour, and knee-shaped at their lower end.

9. OPHIOBOLUS ACUMINATUS

The spores released by the fruit bodies which grown on dead stalks of herbaceous plants might be better considered as half-spores: the brownish,

long, cylindrical, septate spores namely split in their middle and latest at their release. Both parts are now cylindrical, and last but one cell at one end becomes spherical. The species is saprophytic.

10. OPHIOBOLUS ANGUILLIDES

The occurrence of its spores in the air samples is interesting. Its spherical fruit bodies were first found in Hungary by S. TÓTH (1989) on the stalks of Melilotus officinalis L. no more than half a year before the spores appeared for the first time in the air samples in Hungary (Plate I/ Fig. 4).

11. OPHIOBOLUS FRUTICUM

This supposedly saprophytic fungus lives on dead branchlets of Ononis spinosa L.

12. OPHIOBOLUS SPP.

Some times several still not completely developed scolecospores was observed.

13. PARAPHAEOSPHAERIA MICHOTII

This saprophytic fungus is found maidly on dead leaves and stalks of Gramineae and Juncaceae (Plate II/Fig. 6).

14. PHAEOSPHAERIA VAGANS

This supposedly saprophitic fungus lives on the dead stalks of herbaceous plants. Because of its conidial form it belongs to the Phaeosphaeria genus, in which all species generally have spores with only transvers septa, although on the basis of they structure they should belong to the genus Pleospora. They are easily identified by the slender form and the size of their spores.

15. PHAEOSPHAERIA SPP.

Their brown boat shape spores with three or more septa were the most frequently occurring Ascomycetes spores in our samples. Most Phaeosphaeria species are the parasites of Gramineae and Juncaceae.

16. PLEOSPORA SPP.

This genus is, because of their colour and the articulation of its spores very similar to the spores of the Cucurbitaria (and also to some other, in our air samples infrequently found genera like Phaeosphaeria), therefore, their identification could cause difficulties. The conidial form of the most frequently found species, of Pleospora herbarum (Pers.) Rabenh, is the commonest species of Stemphylium (Stemphylium botryosum Wallr.). Its conidial form is not infrequent in the air samples. The fungus is both saprophytic and parasitic (Plate II/Fig. 5).

17. PLEUROCERAS CRYPTODERIS

This saprophytic fungus lives on dead leaves of Populus alba L. Interestingly, we found its spores in the air samples while the fungus itself has this far never been found in Hungary on its natural substrate Populus alba.

18. QUATERNARIA SP.

Its allantoid, brownish, conspicuously big spores are seldom present in air samples. Supposedly a saprophytic fungus, lives on thinner, dead branches of trees (Plate II/Fig. 7).

19. REBENTISCHIA UNICAUDATA

A saprophytic fungus living on decaing stems of herbaceous plants. The lower tapering end of the brown, drop-shaped spores has a colourless appendix.

20. SPORORMIELLA SP.

The spores of these mostly coprophylic species are blackish, on the whole cylindrical, articulated by three or more deep incision, having a visible longitudinal or diagonal germ slit on some of their merospores.

21. XYLARIA SP.

The stroma of these species which mostly develops on decaying stumps, measure several centimetres in height. They are cylindrical, clubshaped, simple or ramified. Saprophytic species; the spores are often hard to distinguish from the similar spores of Hypoxylon.

22. ALTERNARIA SP.

The characteristic obcalvate conidia are arranged in chains of different length on the conidiophores. The conidia are always linked by their thicker ends onto the tapering or break-shaped end of the older member of the conidial chain. The majority of the species in this genus are plant pathogens (Plate III/Figs 12—13).

23. ASTEROSPORIUM ASTEROSPERMIUM

This fungus develops its conidia on the dead branches of Fagus sylvatica L. Its occurrence in our samples refers to a travelling through the air over a distance of several tens of kilometres.

24. CERCOSPORA SP.

The species of Cercospora are common and widespread pathogens of trees and herbaceous plants. About seventy species occur in Hungary.

25. CLADOSPORIUM SPP.

Very common (and sometimes the only genus in the air samples. On account of their ramifying conidial chains they are relatively easily identifiable. The number of species is large, the identification of the conidia to species level is not reliable without cultivation. The conidia are released from

autumn to early summer from dead plant parts. Some of the parasitic species live on specific host(s), especially in the tropical and subtropical zones. In the temperature zones the saprophytic species are more numerous the most common ones being Cl. herbarum Pers. (Link ex S. F. Gray) and Cl. macrocarpum Preuss, the fewer parasitic ones live on many hosts.

26. CURVULARIA SP.

These mostly parasitic species are not frequent in air samples. Their brown, squat conidia are swollen on one side, or a little bent, and have a few septa (Plate IV/Fig. 14).

27. DIPLOCLADIELLA SCALAROIDES

The occurrence of its conidium is very interesting indeed. ARNAUD (1953) who described it first, gave no details about its habit. S. TÓTH collected its conidia first from the foam of a very small river in the Bükk Mountains, Hungary (first Hungarian date); another time he noticed that on a decaying piece of the root of Fumana species developed enormous masses of Diplocladiella scalaroides in a humid chambre. It is a generally thought to be one of the waterfungi (Hypomycetes). Its occurrence in the air makes appear this rather questionable.

28. DRECHSLERA SPP.

It is a frequent parasite of the Poaceae, among others of cereales causing visible dead spots on leaves and all the green parts of the host (Plate IV/ Fig. 14).

29. EPICOCCUM PUPURASCENS

Their characteristic spherical conidia are found in the air mainly from autumn to early summer. They are frequent on dying plants, often causing a conspicuous red spot on them.

30. ERYSIPHACEAE SPP.

The fruit bodies are seldom found in the air (though this might occur) because fruit bodies owing to their characteristically formed appendices get very quickly attached to all kind of things. On the other hand, the conidia, building short chains, are numerous in the air. Being first cylindrical, they later become characteristically "barrel-shaped". After having been released from the chain, the conidium is slightly yellowish.

31. STEMPHYLIUM SP.

The slightly stretched or nearly spherical, longitudinally as well as transversally septate conidia are infrequent but characteristic figures of air samples. Most of the known species are plant pathogens; the more frequent Stemphylium botryosum (an anamorph of the Pleospora herbarum), is a cosmopolitan, perhaps saprophytic fungus.

32. BOLETUS SP.

Mycorrhizal fungi occurring in deciduous as well as in coniferous forests. The smooth, brownish, somewhat elongated spores are infrequently observed.

33. LACTARIUS SP.

Mycorrhizal fungi; the spores are spherical, colourless, with network-like surface, infrequently observed. Over seventy species are known in Hungary.

34. PUCCINIA SP.

The dicellate teleutospores are relatively big, they often have a handle on their lower end. This is the most common genus of the parasitic rust fungi that live on green parts of herbaceous plants.

35. UROCYSTIS SP.

The parasitic fungus is the easiest to identify among all the smut fungi (Ustilaginales). The brown central part of the smut spore consisting of a few cells is surrounded by an incomplete colourless sheet of dead cells.

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THE COMPOSITION OF THE RHIZOPLANE MICROBIOTA OF SESSILE OAK (QUERCUS PETRAEA)

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The frequency of occurrence of bacterial and actinomycete species in samples of fine root fractions of three not damaged and one decaying, 53 years old tree specimens of sessile oak were studied using the root wasing procedure and the dilution plate method for isolating strains from root macerates. A total of 2080 isolates were obtained, from among which 251 representative strains have been selected on the basis of a tentative grouping of similar isolates for detailed studies. Among these strains 39 taxa at species level were separated using conventional differential-diagnostic and numerical taxonomic methods. The most frequently occurring and in all root samples of the four tree specimens detected root partners were _Bacillus brevis_, _Bacillus firmus_, _Klebsiella planticola_, _Streptomyces flavovirens_, _Str. nigrifaciens_, _Str. prunicolor_ and _Str. rutgersensis_. Besides _Erwinia_ spp., _Arthrobacter_ sp., _Str. galbus_, _Str. griseolosuffuscus_ and _Str. spheroides_ proved also to be frequent colonizers but not on the roots of all studied trees. In contrast to the not damaged tree specimens, the rhizoplane microbiota of the decaying tree showed a considerably lower level in species diversity: the number of detectable species diminished with about 50%. New species in this simplified root microbial community has not been detected. In the future further studies will be necessary, but our data make probable, that the change in the root community structure reflects only the existence of certain damaging influences but this change itself is not identical with the latters.

Introduction

During the last two decades a considerable research work was concentrated on a series of supposed damaging environmental factors, considered as more or less responsible causes for the widespread decay in many sessile oak stands, distributing among others in the Middle European Region and within this also in Hungary (JAKUCS 1988; KLEIN and PERKINS 1987; OLESKYN and PRYZBYL 1987; SCHÜLT and COWLING 1985; MÉSZÁROS 1984, etc.). Among the supposed damaging agents microorganisms and viruses were also studied and their possible role discussed (GULDEN and HOILAND 1985; KOSTKA et al. 1984; NIENHAUS 1985; VAJNA 1986, etc.). The aim of our below presented studies was to contribute to our at present very poor knowledge on the composition of

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the indigenous rhizoplane bacterial and actinomycete populations of sessile oak. Because many workers expressed the opinion, that the decay of oak forests can most probably be trace back to the interrelationships of numerous environmental and inner factors, a such complex approach to this important problem is hardly realizable without enough information on the normal root biota.

Material and Methods

Sampling site: Samples of aseptically collected and separated fine root fractions of three healthy (Nos 17, 29 and 30) and one decaying (No. 213) 53 years old sessile oak tree specimens were obtained in October 1990 at the Experimental Station of the Hungarian Forest Institute in the Mátra mountains (N. Hungary) in a partly damaged mixed forest stand of sessile oak and hornbeam located on ranker-type soil.

Isolation of the root bacteria and actinomycetes: With sterile scissors and forceps collected fine roots were washed in Erlenmayer Flasks on a shaker with more than 20 changes of sterile water. After removing the soil particles the roots were macerated by grinding in sterile mortar and from the obtained root paste dilution series was prepared and plated onto starch yeast extract-, peptone beef extract-, soluble starch casein- and synthetic starch ammonium sulphate-agar media. Colonies were isolated on a randomized manner onto slants of identical chemical composition with that of the isolation media.

Selection of representative strains: First of all, the obtained isolates were compared, at cultivating them simultaneously on media of identical composition, studying their cultural-morphological properties, abilities to grow on synthetic and/or complex media, to produce distinguishing pigments, etc. The similar isolates were tentatively grouped together and from every group representative strains were selected for further detailed studies. The number of the strains selected from the individual groups correlated to the number of isolates involved in the group of question.

Description and identification of actinomycete strains: With the exception of a single isolate all of the obtained root actinomycetes proved to be the members of the genus Streptomyces. The representative strains were purified by reisolations and later during their studies continuously checked for purity. Among others the ISP (International Streptomyces Project) differential diagnostic criteria and proposed methods (SHIRLING and GOTTLIEB 1966, 1968, 1969, 1972; SZABÓ and MARTON 1976) were used for their description and their systematic identification at species level were carried out on the basis of the works of WILLIAMS and his collaborators (989) furthermore the determining keys of PRIDHAM (1974) and SZABÓ et al. (1975) were taken also into consideration.

Description and identification of bacterial strains: They were purified by repeated reisolations and checked for purity during the testing period by micromorphological observations and selected physiological tests. Pure cultures of strains were studied and tested for Gram staining; production of endospores; acid fast staining; motility; electronmicroscopic cell morphology; micromorphological changes during the life cycle; colony morphology and pigmentation on different media; soluble pigment production; fermentative and oxidative breakdown of glucose; anaerobic growth; production of catalase, oxidase, phosphatase, DNase, RNase; tolerance to 0-11% NaCl; moist heat resistance at 60 ^{o}C and 80 ^{o}C/30', 60', 90', 120' and 240', respectively; growth at 4, 10, 37, 40 and 45 ^{o}C, respectively; growth at pH 3, 5, 7, 9 and 11, respectively; methyl-red and Voges-Proskauer reactions; growth on Simmon's citrate medium and on MacConkey's-agar; nitrate reduction; methylene-blue reduction; antibiotic activities against Escherichia coli, Bacillus subtilis, Aspergillus niger and Saccharomyces cerevisiae; melanoid pigment production on iron peptone- and tyrosine-agar media; H_2S production from peptone; acid production from arabinose, dulcitol, fructose, galactose, glucose, inositol, inulin, lactose, maltose, raffinose, sorbose, sucrose, trehalose, xylose; decomposition of

cellulose; phenylalanine deamination; indole formation; hydrolysis of urea, casein, starch, gelatin, aesculin, arbutin, Tweens-20, -40, -60, -80, -85; utilization as sole sources of carbon: arabinose, dextrin, dulcitol, fructose, glucose, inositol, inulin, lactose, maltose, mannitol, raffinose, sorbitol, sucrose, furthermore Na-acetate, Na-benzoate, Na-citrate, Na-gluconate, Ca-lactate, Na-malonate, Na-oxalate, Na-pyruvate, Na-salicilate and Na-tartarate; sensitivity to ampicillin (20 µg), carbenicillin (50 µg), chlortetracycline (30 µg), chloramphenicol (30 µg), clindamycin (10 µg), colistin (20 µg), erythromycin (10 µg), gentamycin (20 µg), kanamycin (30 µg), lincomycin (10 µg), neomycin (100 µg), nitrofurantoin (30 µg), nystatin (100 ug), oleandomycin (30 µg), oxacillin (10 µg), oxytetracycline (30 µg), penicillin (3 IU), polymyxin-B (15 µg), spiramycin 30 µg), streptomycin (30 µg), sumetrolim (25 µg), superseptyl (400 µg), tetracycline (30 µg) and vancomycin (50 µg). All of these above listed tests were carried out using internationally accepted methods.

The individual small similarity groups of representative bacterial strains selected from the larger similarity groups of isolates were considered as taxonomic units at species level. These groups have been identified using conventional taxonomic methods with the help of the generic and species descriptions in the latest edition of the Bergey's Manual (1984—1989). The coherence of the members of the individual groups and the validity of the separation of these groups one from another were checked and partly corrected by computer aided numerical taxonomic methods too: the similarity indexes were calculated and a dendrogram was prepared on the basis of 169 coded features.

Results and Discussion

The summarized results of our studies are presented in Table 1. As can be seen altogether 251 representative strains of bacteria and actinomycetes belonging to 41 different groups were separated from among the 2080 isolates, which have been obtained from the rhizoplane of sessile oak. Among these two groups (Nos 14 and 41) comprising 3+24 strains were established by us as heterogeneous assemblages of solitary strains. Thirty-nine relatively homogeneous groups of similar strains we considered as 39 different taxa at species level. Consequently, the rhizoplane population of sessile oak is relatively complex. It is composed of a series of Streptomyces and bacterial species. The above emphasized correlation between the number of representative strains within the individual groups and the number of similar isolates from among which they have been selected makes the approximate evaluation of the frequency of the individual species in the root community possible. E.g. Bacillus brevis, Bacillus firmus, Kelbsiella planticola, Erwinia spp., Arhtrobacter sp., Streptomyces flavovirens, Str. galbus, Str. nigrifaciens, Str. prunicolor and Str. rutgersensis were the most common root partners of sessile oak. SZABÓ (1974) showed that in the rhizoplane of black locust the members of the genus Bacillus can play an important role and according to ALEXANDER (1961) Bacillus brevis is a species, which is generally more common on plant roots, than outside of the rizosphere zone.

Table 1

Distribution of 251 selected and thoroughly studied strains of separated species, genera as well as unidentified groups of bacteria and streptomycetes isolated from the rhizoplane of sessile oak (Quercus petraea) trees of different state of health. Abbreviations: w.s.p.p. = = without symptoms of pathological processes; d.p.d. = decaying processes unambiguously detectable

Group No.	Number of strains	Taxa	Designation of the numbered tree individuals and their visible condition			
			17. w.s.p.p.	29. w.s.p.p.	30. w.s.p.p.	213. d.p.d.
1.	6	Micrococcus luteus	3	1	-	2
2.	3	Micrococcus varians	-	-	1	2
3.	21	Bacillus brevis	5	9	3	4
4.	3	Bacillus brevis var.	1	2	-	-
5.	15	Bacillus firmus	3	2	5	5
6.	14	Klebsiella planticola	3	4	5	2
7.	5	Erwinia sp. I. (Carotovora gr.)	4	-	1	-
8.	2	Erwinia sp. II.	-	-	2	-
9.	4	Red, Gram-neg., catalase-neg., fermenting rods	-	4	-	-
10.	2	Gram-neg., non-motile, non-fermenting rods and filaments	-	2	-	-
11.	4	Gram-positive, fermenting rods	1	2	1	-
12.	15	Arthrobacter (urea-faciens)	12	3	-	-
13.	4	(Microbacterium)?	-	1	3	-
14.	3	A heterogeneous group of bacterial strains	1	-	2	-
15.	1	Str. albohelvatus	1	-	-	-
16.	3	Str. antibioticus	-	2	1	-
17.	3	Str. arenae	2	1	-	-
18.	2	Str. californicus	2	-	-	-
19.	9	Str. cirratus	4	-	2	3
20.	1	Str. citreofluorescens	-	1	-	-
21.	1	Str. erythraeus	-	1	-	-
22.	11	Str. flavovirens	5	1	4	1
23.	10	Str. galbus	1	4	-	5
24.	6	Str. griseolosuffuscus	2	2	2	-
25.	4	Str. halstedii	1	1	2	-
26.	2	Str. humidus	1	-	-	1
27.	1	Str. katrae	1	-	-	-
28.	3	Str. lincolnensis	-	2	1	-
29.	8	Str. nigrifaciens	2	1	2	3
30.	2	Str. nogalater	2	-	-	-
31.	2	Str. pilosus	2	-	-	-
32.	28	Str. prunicolor	6	3	3	16
33.	1	Str. gramulosus	-	1	-	-

Table 1 (cont.)

Group No.	Number of strains	Taxa	Designation of the numbered tree individuals and their visible condition			
			17. w.s.p.p.	29. w.s.p.p.	30. w.s.p.p.	213. d.p.d.
34.	2	Str. rubiginosohelvolus	-	1	1	-
35.	4	Str. rutgersensis	-	4	-	-
36.	13	Str. rutgersensis subsp. castelarense	2	1	2	8
37.	2	Str. spectabilis	-	-	2	-
38.	3	Str. spheroides	-	-	3-	-
39.	1	Str. tuirus	-	1	-	-
40.	3	Str. vastus	-	2	1	-
41.	24	A heterogeneous group of Streptomyces strains	2	7	11	4
	251	(39)	69(23)	66(27)	60(21)	56(12)

Remark: in parentheses the numbers of separated homogeneous groups (species, genera, etc.) of strains are given.

It is a well known fact, that Klebsiella planticola and Erwinia spp. are frequent partners of higher plants and arthrobacters are common members of root bacterial communities. Vegetative, growing actinomycete hyphae are also frequently detectable on root hairs of trees (SZABÓ 1974).

The majority of the 39 groups of strains showed considerable intra-group homogenities. E.g. all of the 28 Str. prunicolor strains proved to be D-galactose, D-xylose, L-arabinose, L-rhamnose, D-fructose, raffinose, D-mannitol and i-inositol positive, salicin negative and only sucrose variable. Differences in their rectiflexibiles sporophore and smooth spore morphology, red spore mass colour, not distinctive soluble and endopigments etc. were not detected and none of them did show melanoid pigment production on peptone iron- or tyrosine-agar. All of the five Erwinia sp. strains of group 7 grew as straight Gram-negative, motile facultatively anaerobic catalase positive, oxidase negative rods capable of glucose fermentation. They grew well on acetate (with one exception), pyruvate and lactate but not on benzoate and oxalate. All of them multiplied at 37 ^{o}C but only two strains at 40 ^{o}C. Their acetoin, urease, indole and phosphatase production was negative. All of them liquefied gelatine, showed positive phenylalanine deaminase reaction, tolerated (with the exception of one strain) 5% NaCl, produced acid from maltose but not from raffinose, inulin, inositol, dul-

citol, suctose and lactose. They utilized as sole source of carbon: arabinose, xylose, lactose and glucose. They did not require growth factors and showed increased sensibilities against moist heat treatments. None of them showed antibiotic activities against the employed test organisms. They tolerated high pH-values and grew on MacConkey's-agar, etc.

The number of isolated species (separated groups of strains) was in the cases of the three healthy tree specimens (Nos 17, 29, 30), 23, 27 and 21, respectively, while in the case of the decaving tree No. 213 only 12. Besides we stated, that only a few species of bacteria (e.g. Bacillus brevis and Klebsiella planticola) and streptomycetes (e.g. Streptomyces flavovirens and Streptomyces prunicolor) occurred in the root region of all of the four studied tree individuals. It is also important, that in the root zone of the dying tree (No. 213), we did not find such aerobic or facultatively anaerobic bacterial or actinomycete species, which were not detected at all, on the roots of one or another, of the healthy oak individuals. Further studies are still necessary, and on the basis of these findings it could only be stated, that in the oak rhizoplane, during the decaying process the number of the coexisting microbial species is considerably decreasing and the complexity of the indigenous root microbiota radically diminishing. Within this, in number of species poor became community however new members, which would be perhaps responsible for induction of the decay have not appeared.

Members of groups 9-10 and 13 remained taxonomically undetermined also at generic level. Bacteria of groups 9, 10 and 11 were relatively frequent on the roots of tree No. 29 while the members of Group 13 in the root zone of tree No. 30. About their taxonomic position we will publish later and elsewhere.

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LIST OF MACROFUNGI PROPOSED FOR PROTECTION IN HUNGARY

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Several species of living organisms are under protection in Hungary but fungi. Although, unfavourable anthropogenic influences do not avoid macrofungi either, and a list of these types of organisms is not any more missing from the Red Data Books published currently in several European countries. The main goal of the present work is the presentation of a list of endangered macrofungi in Hungary. The list reflects our present knowledge about the species to be protected most urgently and about the categories into which they have to be classified for the sake of their successful protection.

Introduction

Increasing anthropogenic influences affect harmfully natural ecosystems, and a growing number of species becomes endangered. Thus, for about two decades, all over the world so-called Red Data Books and Red Data Lists have been compiled, which specify taxone threatened by extinction and outline the possible ways of their protection.

Fungi have been simply omitted of the nature conservation measures and Red Data Lists for a long time (RAKONCZAY 1990). In Europe the supplementation of this insufficiency began in the eighties and macrofungi were also registered in some Red Data Books (WINTERHOF and KRIEGLSTEINER 1984; WÖLDECKE 1987; SCHMID 1990). In Hungary in the last years some works were also published about the possibilities of the protection of macrofungi (BABOS 1989; RIMÓCZI 1992a). RIMÓCZI (1992b) in his article examined the present situation in the country and set the most important duties to be done. The aim of the present work is to complete such a Red Data List of macrofungi, which can be immediately submitted to the officials who are authorized to take the necessary legislative measures.

The proposed list was made by the contribution of several Hungarian mycologists (L. ALBERT, M. BABOS, G. BOHUS, Z. BRATEK, Cs. LOCSMÁNDI, I. RI-

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MÓCZI, I. SILLER, G. VASAS), who determined the endangered categories to be applied to the Hungarian conditions, discussed the possible sources of endangerment as well as the standardized aspects necessary for the selection for protection. The above-mentioned mycologists feel it necessary to carry out aimed researches, which would assess so far mycologically less investigated areas as well as the exact distribution of several species.

The (still by no means accomplished) list published hereafter represents the first stage of an attempt at rescue of endangered macrofungi in Hungary. The list reflects our present knowledge about the species to be protected most urgently and about the categories into which they have to be classified for the sake of their successful protection.

Recommended red data list of macromycetes in Hungary

1. Species endangered by extinction, vulnerable and rare species

There three categories generally accepted in the international literature were fused because of the insufficiency of the data available. Macrofungi becoming extincted and very rare fungi living in extremely endangered biotopes belong to the first group: macrofungi living in strongly endangered biotopes as well as withdrowing and in some areas extincted species into the second group: at the present time still not rare, however, in some areas sporadic, and generally in endangered biotopes living macrofungi as well as those having very special ecological demands belong to the third group. These three, fused groups are recommended for protection. The species are as follows:

AGARICALES

Agaricus bernardiiformis Bohus
A. macrosporoides Bohus
Amanita caesaria (Scop.: Fr.) Pers.: Schw.
A. solitaria (Bull.: Fr.) Mérat
A. vittadini (Mor.) Vitt.
Aureoboletus cramesinus (Secr.) Sing.
Boiletus dupainii Boud.
B. feuchtneri Vel.
B. regius Pers.: Fr.

B. rhodopurpureus Smott

B. rhodoxanthus Krombh.

B. torosus Fr.

Camarophyllus lacmus (Schum.) Lge.

Chalciporus rubinus (W. G. Smith) Sing.

Gomphidius roseus (Fr.) Fr.

Hebeloma ammophilum Bohus

Hebeloma ochroalbidum Bohus

genus Hygrocybe: all species in Hungary are to be protected

Leccinum holopus (Rostk.) Watl.

L. variicolor Watl.

Limacella ochraceolutea P. D. Orton

Oudemansiella nigra Dörfelt

Phyllotopsis nidulans (Pers.: Fr.) Sing.

Pleurotus eryngii (DC.: Fr.) Quél. var. ferulae Lanzi

Rhodotus palmatus (Bull.: Fr.) Mre.

Squamanita schreieri Imbach

Suillus placidus (Bon) Sing.

Tricholoma atrosquamosum (Chev.) Sacc.

T. aurantium (Schiff.: Fr.) Ricken

T. bresadolianum Clc.

T. goniospermum Bres.

T. focale (Fr.) Ricken

T. pardinum Quél.

T. sulphurescens Bres.

Volvariella bombycina (Schiff.: Fr.) Sing.

V. surrecta (Knapp) Sing.

Xerocomus parasiticus (Bull. Fr.) Quél.

GASTEROMYCETES-ASCOMYCETES

Anthurus archerii (Berk.) Fischer

Elaphomyces maculatus Vitt.

E. virgatosporus Holl.

Montagnea radiosa (Pallas) Sebek (syn. M. arenaria)

Myriostoma coliforme (Dicks. ex Pers.) Corda

Phellorinia inquinans Berk.

Pisolithus tinctorius (pers.) Desv.

APHYLLOPHORALES

genus Albatrellus: all species in Hungary are to be protected
genus Bankera: all species in Hungary are to be protected
genus Hydnellum: all species in Hungary are to be protected
Hymenogaster cerebellum Cavara
Gomphus clavatus (Pers.: Fr.) S. F. Gray
Polyporus rhizophilus Pat.
genus Sarcodon: all species in Hungary are endangered (they are to be increasedly protected)

2. Species to be shielded

In this group were listed macrofungi, which have got endangered because of the mass gathering and selling as well as those species, which are still not rare, but are decaying mycorrhizal companions of forest trees or important representatives of the characteristic fungous flora of Hungary.

AGARICALES

Agaricus bernardii Quél.
A. maskae Pilat
A. bohusii Bon.
Amanita beckeri Huijsm.
A. ovoidea (Bull.: Fr.) Quél.
A. regalis (Fr.) Mre.
Boletinus cavipes (Opat) Kalchbr.
Boletus appendiculatus Schff.: Fr.
genus Cortinarius: all the species having great fruit bodies are to be shielded
Gyrodon lividus (Pers.: Fr.) S. F. Gray
Hygrophorus russula (Schff.: Fr.) Quél.
genus Inocybe: all species growing on sand are to be protected
genus Lactarius: all species are to be protected with the exception of L. piperatus (L. ex Fr.) S. F. Gray, L. pargamenus (Swartz ex Fr.) Fr., L. deterrimus Gröger, L. deliciosus Fr., L. sanguifluus (Paulet ex Fr.) Fr. and L. semisanguifluus Heim et Lecl.
genus Leucoagaricus: species growing on sand are to be protected
genus Leucopaxillus: all species are to be protected
Limacella guttata (Pers.: Fr.) Konr. et Maubl.

Pleurotus calyptratus (Lindbl. ap. Fr.) Sacc.

P. dryinus (Pers.: Fr.) Kumm.

genus Psathyrella: species growing on sand are to be protected

Ripartitella rickeni (Bohus) Sing.

genus Russula: all species are to be protected with the exception of Russula cyanoxantha Schff. ex Fr., R. heterophylla (Fr.) Fr. and R. vesca Fr.

Strobilomyces floccopus (Vahl in Fl. Dan.: Fr.) Karst.

Suillus aeruginascens (Secr.) Snell

Tricholoma orirubens Quél.

GASTEROMYCETES—ASCOMYCETES—APHYLLOPHORALES

Battarea phalloides (Dick.) ex Pers.

B. stevenii (Liboschitz) Fr.

Endoptychum agaricoides Czernajev

genus Geastrum: all species are to be shielded

Polyporus tuberaster (Pers.) ex Fr.

genus Tulostoma: all species are to be protected

Tuber aestivale Vitt.

For the sake of the protection of macrofungi in Hungary we think it necessary to take the following measures:

— The endangered macrofungi in the nature reserves, landscape protection areas as well as national parks should be also in practice protected (the gathering of the mushrooms in these areas must be prohibited with the exception of sellable species).

— The macrofungus species suggested for protection should not be permitted to sell.

— The macrofungi listed in the category of species to be shielded should be gathered only moderately.

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GROWTH CHARACTERISTICS AND BIOSYNTHETIC ABILITIES FROM CALLUS CULTURES OF THREE CHEMOTYPES FROM DATURA INNOXIA MILL.

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Callus cultures from three ecotypes of Datura innoxia were initiated. The cultures were initiated from stem segments of aseptically grown seedlings and cultivated under constant conditions for approximately 18 months. During that time the cultures studied in the present investigation showed distinctive growth characteristics and different abilities for tropane alkaloid accumulation. However, abilities for expression of tropane alkaloid pattern could not be related to the growth behavior and phenotypical characteristics of the cultures.

Introduction

Cell and callus cultures of tropane alkaloid producing plants, believed to be convenient system for rapid biomass production, have been widely studied for their ability to produce secondary compounds. Tropane alkaloid accumulation in such cultures was found to be quire pure (MISSALEVA et al. 1993; SZŐKE et al. 1982, 1984; VERZÁR-PETRI et al. 1981, 1982; YAMADA and HASHIMOTO 1982). Alkaloid patterns of unorganized cultures initiated from plant species belonging to the genus Datura significantly differed from those of parent plants (STABA and JINDRA 1968). Nor changes in cultural conditions neither addition of tropane alkaloid precursors or different growth regulators affected significantly alkaloid accumulation in cultures of several Datura species (HIRAOHA 1976; NGUYEN N. DUNG et al. 1981; POTOCZKI et al. 1982; STABA and JINDRA 1968; VERZÁR-PETRI et al. 1980). Although several reports had been conducted on the secondary procution of unorganized

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cultures of plant species, known as producers of tropane alkaloids, no data are available for biosynthetic activity of such cultures in case of prolonged cultivation. In the present investigation callus cultures of three ecotypes of Datura innoxia, cultivated under similar conditions, had been assayed for their biosynthetic activity for almost 18 months cultivation period.

Material and Methods

Initiation of callus cultures

Seeds of Datura innoxia Mill., obtained from a variety of botanical gardens (392-Geneva, 228-Tries, 903-Amsterdam) were germinated on solid Murashige—Skoog's (MS) (MURASHIGE and SKOOG 1962) nutrient medium, supplemented with 0.05 $mg \cdot l^{-1}$ kinetin, 0.05 $mg \cdot l^{-1}$ NAA (α-naphthaleneacetic acid), 0.05 $mg \cdot l^{-1}$ GA_3 (gibberellic acid), 200 $mg \cdot l^{-1}$ myo-inositol, 200 $mg \cdot l^{-1}$ casein hydrolysate, 200 $mg \cdot l^{-1}$ yeast extract, 0.5% (w:v) D-glucose and 1.5% (w:v) sucrose. Stem segments of resultant seedlings were employed for initiation of callus cultures. For callus induction and maintenance of resulting callus cultures MS nutrient medium, supplemented with 0.5 $mg \cdot l^{-1}$ NAA, 0.1 $mg \cdot l^{-1}$ kinetin and 500 $mg \cdot l^{-1}$ casein hydrolysate was used. For the sake of clarity the initiated callus cultures were designated under the number shown above. The cultures were cultivated under luminescent light — 2500 lx, 12 h/day at 25 °C.

At the end of each cultivation period (25 days) the fresh and dry weights in five replicates were determined. On the basis of these measurements dry matter contents were calculated.

Extraction and purification of alkaloids

Freeze-dried plant samples were powdered and extracted with NH_4OH-10% (v:v)/$CHCl_3$ (1:9) mixture in sonicating bath (Tesla Vrable, k.p.; UC 002 BM1) for 20 min. The combined extracts of three subsequent extraction procedures were evaporated to dryness and the dry residues were collected by washing with 2% (v:v) H_2SO_4. The acidic-aqueous solutions were made alkaline (pH 8-9) by adding NH_4OH (20%) and extracted three times with $CHCl_3$. The chloroform fractions dried by filtering through anhydrous Na_2SO_4 were evaporated to dryness and the residues, dissolved in appropriate quantities of $CHCl_3$ were used for the TLC identification and determination by comparison with standards of hyoscyamine, scopolamine and cuscohygrine. The TLC separation was carried out on silica-gel 60 F_{254} Merck plates. The chromatograms were developed by using 0.2 M NaAc ($CH_3OH/CHCl_3$) n-hexan (1:6:3:1) as a mobile phase. The alkaloid spots were located by Dragendorff's reagent (MUNIER and MACHEBOEUF 1949) in which the quantity of stock solution was changed to 3 ml. The TLC-densitometry was carried out by using a Shimadzu densitometer, consisting of high speed dual-wavelength scanner CS-930 (Shimadzu, Japan) and DR-2 data recorder at 530 and 660 nm.

The alkaloid determination was performed in three replicates and the standard errors of the means are presented in the subsequent tables.

Results and Discussion

Callus cultures initiated from stem segments of in vitro germinated seedlings of three ecotypes of Datura innoxia were subcultivated until the

Table 1

Alkaloid content characteristics of seedlings of *Datura innoxia* employed for initiation of callus cultures

Ecotype No.	Plant vegetative part	Alkaloid content (% of dry weight)			
		Hyoscyamine		Scopolamine	
		Mean	± SE	Mean	± SE
228	upper parts		-	0.0041	0.0009
	roots	0.0026	0.0015	0.0017	0.0008
903	upper parts		-	0.0030	0.0010
	roots	0.0040	0.0011	0.0030	0.0010
392	upper parts		-	0.0032	0.0011
	roots	0.0015	0.0005	0.0010	0.0005

± SE — Standard error

Table 2

Growth characteristics and alkaloid accumulation in callus cultures of ecotype 903

Passage No.	Fresh weight (g)		Dry weight	Dry matter content	Alkaloid content (% of dry wt./relative)				Accomp. compound
					Hyoscyamine		Cuscohygrine		
	Mean	± SE	(g)	(%)	Mean	± SE	Mean	± SE	hR_F
1	8.4895	0.5262	0.4067	4.7909	—	—	—		—
2	11.5168	0.4693	0.3557	3.0885	—	—	—		—
3	10.6555	0.6349	0.3757	3.5260	—	—	—		—
4	10.2534	0.2277	0.3485	3.3988	—	—	+		—
5	9.4500	0.1915	0.4420	4.6770	—	—	—		—
6	15.9520	0.2874	0.3926	2.4613	—	—	—		—
7	10.4012	0.2032	0.4538	4.3628	—	—	—		—
8	9.8513	0.1671	0.4027	4.0882	—	—	—		—
9	10.3354	0.3831	0.3832	3.7072	—	—	—		—
10	11.3724	0.4017	0.3305	2.9058	—	—	—		—
11	10.1613	0.4559	0.3192	3.1417	0.0008	0.0005	+		Scopol.
12	12.5632	0.4145	0.3055	2.4317	—	—	—		—
13	11.2657	0.2989	0.3199	2.8398	—	—	—		—
14	10.6247	0.3633	0.3166	2.9800	—	—	—		—
15	10.6610	0.3461	0.3313	3.1074	—	—	0.0008	0.0002	—
16	9.7911	0.1977	0.2956	3.0187	—	—	0.0010	0.0005	—
17	8.9862	0.5985	0.4639	5.1622	—	—	+		—
18	9.8223	0.1896	0.3143	3.2000	—	—	—		—

± SE — Standard error

Table 3

Growth characteristics and alkaloid accumulation in callus cultures of ecotype 228

Passage No.	Fresh weight (g)		Dry weight	Dry matter content	Alkaloid content (% of dry wt./relative)				Accomp. compound
					Hyosciamine		Cuscohygrine		
	Mean	± SE	(g)	(%)	Mean	± SE	Mean	± SE	hR_F
1	9.7268	0.2416	0.3307	3.3999	—	—	—		—
2	8.4507	1.0389	0.2987	3.5346	—	—	—		—
3	8.8198	0.3130	0.3269	3.7070	—	—	—		—
4	11.6549	0.6094	0.3750	3.2174	—	—	—		—
5	8.0454	0.5540	0.3391	4.2152	—	—	—		—
6	13.8294	0.8006	0.4028	2.9129	—	—	+		—
7	11.8652	0.3831	0.3020	2.5433	—	—	—		—
8	11.9469	0.3361	0.3520	2.9461	—	—	—		—
9	8.5500	0.6750	0.2772	3.2426	—	—	—		—
10	9.4261	0.5538	0.2900	3.0769	—	—	—		—
11	12.3112	0.7450	0.4198	3.4096	—	—	—		—
12	9.6941	2.3516	0.2592	2.6738	—	—	—		—
13	10.2425	0.8284	0.2807	2.7403	—	—	—		—
14	7.5353	0.5749	0.2659	3.5294	—	—	0.0003	0.0002	75
15	10.7314	1.4926	0.3689	3.4372	—	—	0.0008	0.0004	75
16	9.3904	1.6922	0.2802	2.9844	—	—	0.0003	0.0002	—
17	8.5576	1.0756	0.3629	4.1238	—	—	0.0001	0.0000	—
18	9.9187	0.8610	0.3728	3.7583	—	—	—		—

± SE — Standard error

18th passage. Plantlets used for initiation of callus cultures displayed similar alkaloid content characteristics of their vegetative parts at 4 weeks post-germination development (Table 1).

Callus cultures of the above-mentioned ecotypes initiated and subcultivated under similar conditions quite distinctive from one another in appearance and biosynthetic activity.

Cultures of 903-plants were yellow-white in color and creamy in texture in all passages of subcultivation. Observed growth fluctuations (Table 2) were associated with any changes of organization degree. Tropane alkaloid accumulation was quite pure presented in 903-cultures during their almost 18-months maintenance. The main tropane derivative detected in most of the subcultures was cuscohygrine. It was found in measurable amounts in the 15th and 16th passage only (Table 2). Different alkaloid pattern was observed in the 11th passage of subcultivation. Except for the cuscohygrine both hyoscyamine and scopolamine were detected. Among those hyoscyamine was detected in higher amounts. Although the above-mentioned alkaloids were

Table 4

Growth characteristics and alkaloid accumulation in callus cultures of ecotype 392

Passage No.	Fresh weight (g)		Dry weight	Dry matter content	Alkaloid content (% of dry wt./relative)				Accomp. compound
					Hyoscyamine		Cuscohygrine		
	Mean	± SE	(g)	(%)	Mean	± SE	Mean	± SE	hR F
1	11.3299	0.6088	0.2993	2.6417	+		+		—
2	10.1521	0.3097	0.3838	3.7807	0.0038	0.0006	++		—
3	11.5371	0.4270	0.3373	2.9236	0.0066	0.0016	+		—
4	12.6978	0.8295	0.4955	3.9024	—	—	+		—
5	9.7244	0.4765	0.4706	4.8396	0.0047	0.0006	+++		—
6	11.5305	0.5691	0.2972	2.5778	0.0055	0.0014	+++		—
7	13.0224	1.3388	0.3592	2.7587	0.0112	0.0022	+		—
8	13.5206	0.5196	0.4277	3.1630	—	—	+		—
9	10.7236	0.7125	0.3751	3.4979	0.0069	0.0011	++		—
10	11.6885	1.2949	0.4297	3.6759	0.0095 0	0.0014	++		—
11	13.2085	0.5602	0.4095	3.1006	—	—	+		—
12	11.9232	0.8382	0.5444	4.5659	0.0090	0.0030	+		—
13	9.1982	0.6113	0.2490	2.7057	—	—	0.0008	0.0004	50; 67
14	9.4405	0.6391	0.2908	3.0800	0.0085	0.0020	0.0004	0.0002	—
15	14.6359	0.6867	0.4703	3.2136	0.0092	0.0036	0.0003	0.0002	—
16	13.3083	0.4793	0.3894	2.2964	—	—	0.0095	0.0014	72
17	9.1180	0.3970	0.4139	4.5394	0.0090	0.0028	0.0011	0.0010	18.7
18	13.5472	0.9380	0.3230	2.3845	—	—	+		—

± SE — Standard error

detected in small amounts, their presence gave an evidence for expressed synthesis of the enzyme activities responsible for their formation.

The 228 cultures were green in colour and exhibited aggregation in all passages of subcultivation. No wide variation of callus growth was observed in the subcultures (Table 3). Expression of normal alkaloid pattern was not observed in 228 cultures during their maintenance. Cuscohygrine was the main secondary compound detected in the cultures, but mainly in their late passages.

Callus cultures initiated from 392 plants showed different behavior during subcultivation. In the initial passages they were mainly light yellow-green in colour and exhibited low aggregation. In the 4th passage of subcultivation spontaneous of globular structures took place in those cultures. The cultures preserved ability produce organogenic structures in three subsequent passages. In the later subcultures ability for self-initiation of the above-mentioned structures was suppressed, but cultures became much less friable. Callus cultures initiated from 392 plants exhibited
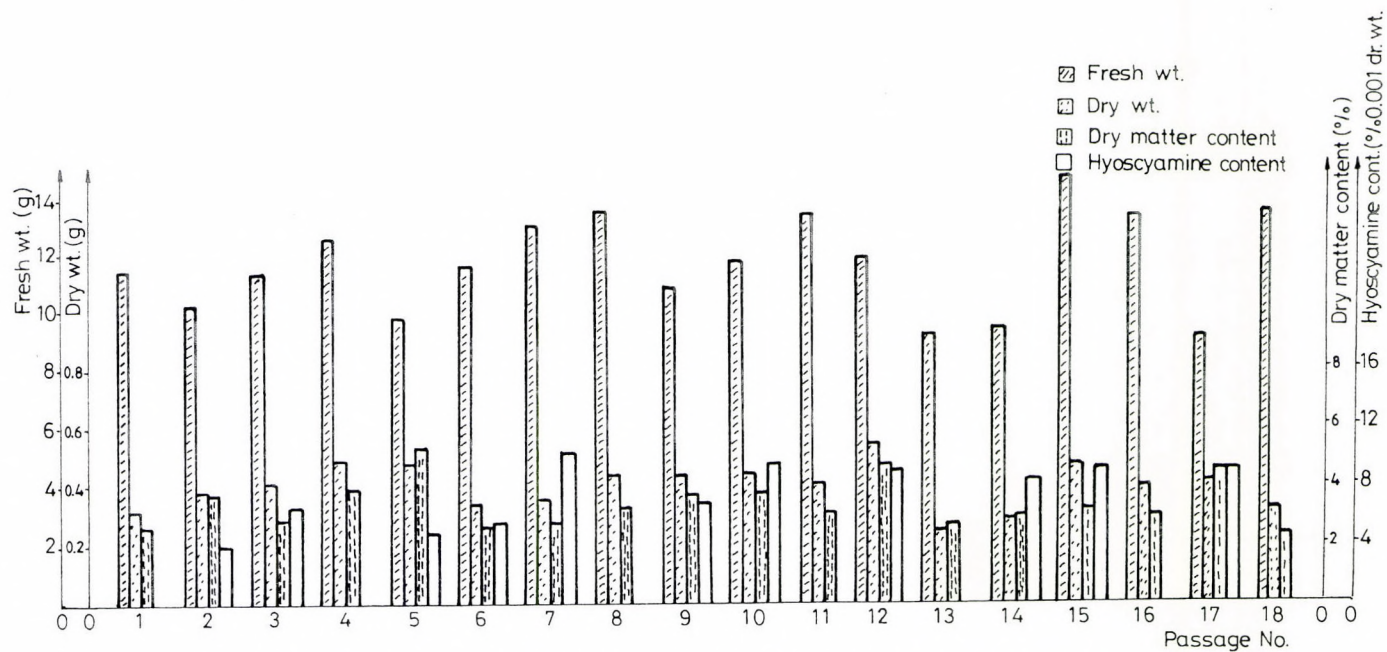
Fig. 1. Growth characteristics and alkaloid accumulation in callus cultures initiated from 392 seedlings

higher biosynthetic ability than that of the above-mentioned ecotypes. The major alkaloid detected in those cultures was hyoscyamine, but its synthesis was not expressed in all subcultures (Table 4). Hyoscyamine was found in amounts comparable or higher than in donor seedlings. In most of the subcultures slowed down growth was accompanied by expression of alkaloid synthesis, but not all growth fluctuations were attended by secondary product accumulation (Fig. 1).

Callus cultures of three ecotypes of Datura innoxia, whose growth response and biosynthetic activities were studied in the present investigation were quite distinctive from one another in their almost 18 months maintenance. Those differences could not be related to the biosynthetic capacities of donor material. In this connection it would be mentioned that similar observations were already made for unorganized cultures of another tropane alkaloid producing plant species (FRANKHAUSER et al. 1986).

No relationship was observed between alkalod composition of plant material, used for explants than that of resultant callus cultures. Hyoscyamine and cuscohygrine were not constitutents of aerial parts of donor seddlings, but they were detected in initiated unorganized cultures. These results were consistent with previous reports for difference in alkaloid patterns of callus cultures and plant parts used for their initiation (STABA and JINDRA 1968; VERZÁR-PETRI et al. 1978). However, the presence of the above-mentioned alkaloids in unorganized cultures indicated that the plant cells retained the genetic information for the biochemical traits of the plants species.

Tropane alkaloid accumulation was better presented in cultures originated from 392 seedlings, but alkaloid synthesis was not expressed in all subcultures. The hyoscyamine was detected in amounts comparable or higher than in donor material. However, its content was lower than in field plants (WITTE et al. 1987; YANKULOV et al. 1979). Low tropane alkaloid accumulation and instability of the alkaloid production are not restricted to callus and cell cultures of genus Datura. This problem exists also in unorganizec cultures of other Solanaceous genera (KURTZ and CONSTABEL 1985; PETRI 1988).

Callus cultures displayed changeable growth characteristics during subcultivation. Observed growth fluctuations could not serve as indication for biosynthetic activity, although in most of the passages of 392 cultures alkaloid accumulation was observed if the growth was relatively slow.

The instability of alkaloid accumulation would be due to reversible biochemical modifications occurring within the cell strains. However, abili-

ty to generate modifications resulting into expression of alkaloid synthesis in unorganized cultures under given conditions seems to be highly genotype dependent.

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SCREENING OF ANTIOXIDATIVE CAPACITY OF WATER EXTRACTS OF HUNGARIAN MEDICINAL PLANTS AND WILD SPECIES

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In the recent advising experiments (subsidised by Hungarian Scientific Research Fund, OTKA-1324) 76 plant-samples were analysed for antioxidative capacity by determining the redox-state potential (E_0') of water-extracts (fluids) at primoridal and human physiological pH (pH 7.4). There was also investigated the effect of boiling on the redox-state potential. Both the pH and E_0' was measured in water solutions.

In the case of cold extracts *Betonica officinalis*, *Rosa canina*, *Dracocephalum moldavicum*, *Populus nigra*, *Allium ursinum*, *Cotinus coggygria*, *Rosmarinus officinalis*, *Origamus vulgare* and *Tilia cordata* drugs had the lowest E_0' at the primordial pH (ph 4.5—5.5) while at pH 7.4 the redosis was increased significantly in the most cases. The boiling had usually decreasing effect on the redosis (the redox-state potential was increased).

Introduction

Recently, there has been increasing interest in oxygen-containing free radicals in biological systems and their implied roles as causative agents in the etiology of a variety of chronic disorders. Accordingly, attention is being focused on the protective biochemical function of naturally occurring antioxidants in the cells of the organisms containing them, and on the mechanisms of their action. Many enzymes (catalase, peroxidases, superoxide dismutase) and secondary compounds of higher plants have been demonstrated in in vitro experiments to protect against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species. The evidence supports at least a partial antioxidant role in vivo for many classes (e.g. tocopherols, flavonoids, phenolic acids, alkaloids, amino acids and amines, chlorophyll derivatives, carotenoids, ascorbic acid) of plant metabolites (LARSON 1988).

There is a very complex relationship between food and oxygen. From this viewpoint, antioxidants in food are in themselves indispensable consti-

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tuents of food (NAMIKI 1990). Lipid peroxidation is known as one of the major factors for deterioration during the storage and processing of foods. In addition, it is considered to induce physiological obstruction causing cell aging or carcinogenesis. A lot of crude drugs prepared from plant materials are traditionally used and their pharmacological effects have been extensively studied from various viewpoints. SU, OSAWA and NAMIKI (1986) dealt with screening for antioxidative activity in 195 species of crude drugs. According to their results eight species showed strong antioxidative activity in the ether or ethyl acetate extracts. These monopolar extracts contained tocopherols.

It is very important fact that many polar fenoloids of plants have antioxidant effect (FARKAS et al. 1985; ADZET 1986; TOREL et al. 1986), e.g. flvan-3-ol derivatives (catechins), the basis structural unit of condensed tannins, are known to possess strong antioxidative activities, and several investigations on the activities of tea or grape catechins have been attempted (MATSUZAKI and HARA 1985; HIROSE et al. 1990). Nevertheless, ZHANG et al. (1990) reported quinone-type compounds from rhizomes of Salvia miltiorrhiza as having strong antioxidative effects.

As is known, the tissue redox-state potential is one of the regulators of physiological processes (PUPPI and DELY 1983). Particularly it is known as well that a shift to oxidosis evokes the damage of cell membranes mostly their lipid components, while a shift to redosis decreases the entropy of the open system will be increased. So it is advantageous to increase the uptake of the quantity of antioxidants. In the earlier experiments we measured the E_0' values of different Indian herbal plants and teas (SZABÓ et al. 1988). Every drug was found to have a characteristic redox-state potential (symbol: E_0'). E_0' dependence of pH was more expressive in the boiled fluids than the cold ones. Redosis increased at pH 7.4. On an average larger redosis was observed in cold fluids as compared to that in boiled decoctions. Therefore, the redox-state potential depends not only on the pharmacologically active substances but on the cytoprotective materials also. It is known that polyphenolic compounds, e.g. flavonoids play a very important role in the antioxidative capacity of different plant species.

In the recent experiments we measured the redox-state potential value of water extracts (fluids) of different herbal and wild species collected in Hungarian flora.

Materials and Methods

The various crude plant material used for this experiment were collected in Mecsek-hill (26 samples), around Pécs and other places (16 samples) and Herbaria — Trade Company of Medicinal Plant, Budapest (34 samples) in 1985—1990. Each finely powdered plant material (3 g) was extracted with 100 ml of distilled water at room temperature by shaking for one hour. During the extraction by boiling (5 minutes) 3 g of material was similarly used in 100 ml of distilled water and filtered.

Redox-state potential (E_0') was measured in the filtrates of the extracts at the primordial pH (4.5—5.5) and physiological pH (for human: pH 7.4). The readings were taken every one minute for 10 minutes using a potentiometric method (CATER et al. 1957). In 10 minutes values were nearly unchanging. Platinum semi-microelectrodes, insulated with enamel except at the tips, were stuck into the test solutions. For reference Ag/AgCl electrodes, immersed in the incubating Ringer solution, were used. Following compensation of the electrode potentials, the value of E_0' was expressed as potential difference between the platinum and silver electrodes. A digital pH meter of high input resistance (Radelkis made in Hungary) was employed.

Results and Discussion

The relative values (mV) of the redox-state potential (E_0') are very different in many instances (Table 1). At the primoridal pH (pH 4.5--5.5) great negative values were measured — the redosis was increased — in fluids of the following species: Betonica officinalis shoot with leaves and flowers (herba or herb), Rosa canina fruit (fructus or pseudofructus), Dracocephalum moldavicum herb, Populus nigra bud (gemma), Allium ursinum bulb (bulbus), Salvia verticillata herb, Cotinus coggygria leaf (folium), Rosmarinus officinalis leaf, Origanum vulgare herb, Tilia cordata flower (flos).

The great plus values were measured — the oxidosis was increased — in fluids of the following species: Fagus sylvatica leaf, Cichorium intybus root (radix), Stachys sylvatica herb, Marrubium vulgare herb, Allium ursinum leaf, Festuca drymeia herb, Equisetum arvense herb, Stachys annua herb, Leonurus cardiaca herb, Datura stramonium herb, Oxalis acetosella leaf.

At pH 7.4 the relative values decreased — the redosis was increased -- in every case, especially e.g. Betonica officinalis, Dracocephalum moldavicum, Origanum majorana leaf, Aesculus hyppocastanum seed (semen), Carpinus betulus leaf, Galium odoratum herb, Linum usitatissimum brown seed, Crataegus monogyna fruit.

The difference between relative values at the primordial and human physiological pH (pH 7.4) expresses the degree of changes in 10 minutes without exception. After 10 minutes the values change hardly.

There was also investigated the effect of boiling on the redox-state potential (Fig. 1). The treatment of high temperature (boiling) causes very

Table 1

Relative values (mV) of the redox-state potential (E_0') of cold fluid (after 1-hour-extraction with water at 25 °C) of drugs and wild plant species collected from Hungarian flora. (Numerical order in increasing of mV-values at primordial pH)

No.	Species	Part of plant (Latin term)	Origin of sample	Primordial pH (mV)	pH 7.4 (mV)	Difference (Δ mV)
				in 10 minutes (E_0')		
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1.	Betonica officinalis	herba	H	-39	-137	98
2.	Rosa canina	fructus	H	-37	-60	23
3.	Dracocephalum moldavicum	herba	H	-28	-131	103
4.	Populus nigra	gemma	H	-27	-57	30
5.	Allium ursinum	bulbus	Mecsek	-24	-24	0
6.	Salvia verticillata	herba	mecsek	-11	-31	20
7.	Cotinus coggygria	folium	Mecsek	-7	-11	4
8.	Rosmarinus officinalis	folium	H	-6	-58	52
9.	Origanum vulgare	herba	Mecsek	-4	-68	64
10.	Tilia cordata	flos	H	-3	-72	69
11.	Ononis spinosa	radix	H	-2	-20	18
12.	Quercus robur	folium	Mecsek	1	-91	92
13.	Vicia faba	semen	Bicsérd	1	-52	53
14.	Pimpinella anisum	fructus	H	3	-24	27
15.	Aegopodium podagraria	herba	Mecsek	8	-70	78
16.	Melica uniflora	herba	Mecsek	9	-37	46
17.	Ambrosia elatior	herba	Bicsérd	12	-53	65
18.	Origanum majorana	folium	H	13	-150	163
19.	Thymus vulgare	herba	H	14	-59	73
20.	Rumex confertus	herba	Mecsek	21	-64	85
21.	Ribes nigrum	folium	H	24	25	1
22.	Salvia officinalis	folium	H	24	-2	26
23.	Corydalis cava	herba	Mecsek	28	0	28
24.	Hypericum perforatum	herba	Mecsek	29	-47	76
25.	Gentiana lutea	radix	H	29	-40	69
26.	Foeniculum vulgare	fructus	H	29	4	25
27.	Aesculus hyppocastanum	semen	Pécs	30	-133	163
28.	Quercus petraea	folium	Mecsek	30	-66	96
29.	Glechoma hederacea	herba	Mecsek	30	-9	39
30.	Carpinus betulus	folium	Mecsek	31	-126	157
31.	Galium odoratum	herba	Mecsek	32	-117	149
32.	Melilotus officinalis	herba	H	34	2	32
33.	Melissa officinalis	folium	H	35	-45	80
34.	Echinochloa crus-galli	herba	Bicsérd	36	-45	81
35.	Satureja hortensis	herba	H	38	-71	109
36.	Hyssopus officinalis	herba	H	38	-58	96
37.	Calendula officinalis	flos	H	39	-56	95
38.	Abutilon theophrasti	herba	Bicsérd	39	-63	102
39.	Carex pilosa	herba	Mecsek	38	-40	78
40.	Mentha piperita	folium	H	40	-65	105
41.	Helianthus annuus	herba	Bicsérd	40	4	36
42.	Abutilon theophrasti	epicarpium	Bicsérd	41	-43	84
43.	Agrimonia eupatoria	herba	H	41	37	4
44.	Allium ursinum	folium	Mecsek	43	34	8

Table 1 (cont.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)
45.	Linum usitatissimum	semen (brown)	Szeged	47	-116	163
46.	Frangula alnus	cortex	H	47	6	41
47.	Glycine soja	legumen	Bicsérd	48	-62	110
48.	Malva sylvestris	folium	H	50	29	21
49.	Agropyron repens	rhizoma	H	51	4	47
50.	Achillea millefolium	herba	H	52	2	50
51.	Salvia nemorosa	herba	Mecsek	54	-63	117
52.	Calluna vulgaris	herba	H	55	1	54
53.	Salvia farinacea	herba	Pécs	56	-84	140
54.	Polygonum lapathifolium	semen	Bicsérd	56	-62	118
55.	Salvia pratensis	herba	Mecsek	57	-78	135
56.	Linum usitatissimum	semen (yellow)	Szeged	57	-46	103
57.	Cerasus avium	stipes	H	58	-12	70
58.	Papaver somniferum	caput	Bicsérd	59	10	49
59.	Crataegus monogyna	fructus	Mecsek	62	-128	190
60.	Quercus cerris	cortex	H	64	-99	163
61.	Lamium album	herba	H	64	17	47
62.	Crataegus monogyna	summitas	Mecsek	64	54	10
63.	Juniperus communis	fructus	H	66	-23	89
64.	Galeopsis speciosa	herba	Mecsek	69	5	64
65.	Artemisia vulgaris	herba	Bicsérd	72	21	51
66.	Fagus sylvatica	folium	Mecsek	77	-78	155
67.	Cichorium intybus	radix	H	77	-38	115
68.	Stachys sylvatica	herba	Mecsek	77	28	49
69.	Marrubium vulgare	herba	H	81	12	69
70.	Allium ursinum	folium	Mecsek	82	52	30
71.	Festuca drymeia	herba	Mecsek	91	-53	144
72.	Equisetum arvense	herba	H	92	47	45
73.	Stachys annua	herba	Bicsérd	93	-5	98
74.	Leonurus cardiaca	herba	H	97	-81	178
75.	Datura stramonium	herba	Bicsérd	105	-17	122
76.	Oxalis acetosella	folium	Mecsek	154	-62	216

H = Herbaria (Trade Company of Medicinal Plant, Budapest)
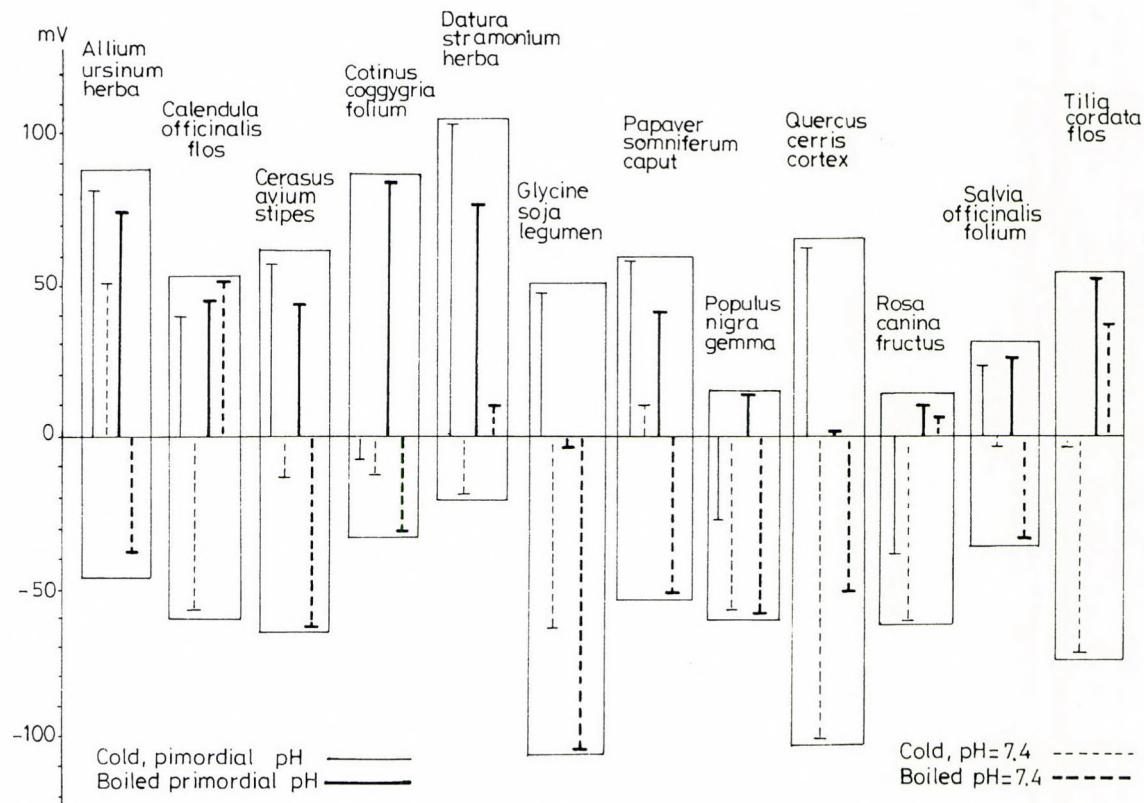
Fig. 1. Effect of boiling on the redox-state potential of some species
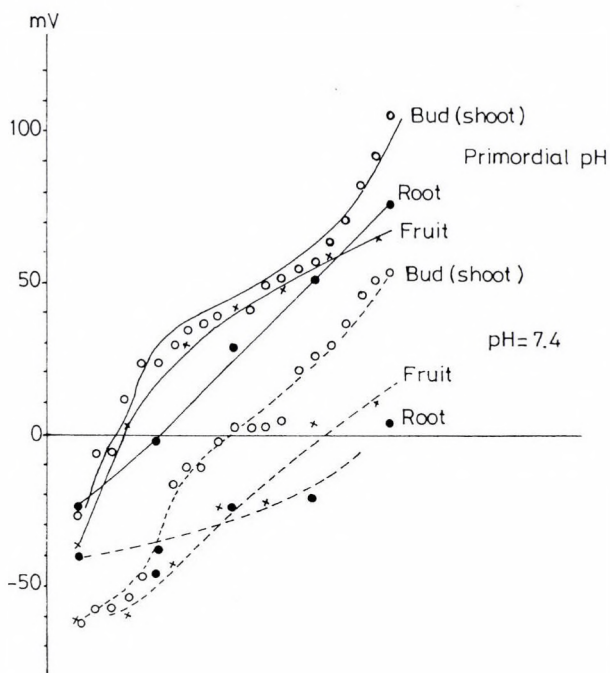
Fig. 2. Partition of values according to different parts of the plants

different change according as what kind of part of the plant was used for boiling. Generally the boiling had a decreasing effect on the redosis, namely the redox-state potential increased especially at primordial pH.

Increasing pH had a significant dereasing effect on redox-state potential almost in every case, namely increased the redosis (Fig. 2). This effect seems to bear a relation to the taxonomic place of species (Fig. 3).

Consequently, the changes of pH and temperature may cause many kinds of process in water extracts of plants (e.g. polimerization, precipitation, oxidation, etc.). However the degree of this change may depend on physiological state of the plant or the part of plant, namely the time of gathering samples. Nevertheless, our data give information on the effect of the pH and high temperature treatment on the redox-state potential as a parameter of the antioxidant character.
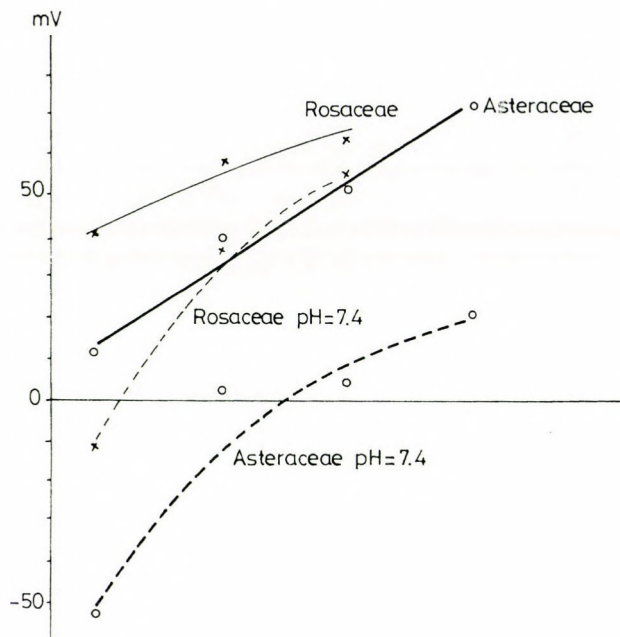
Fig. 3. Values of redox-state potential are characteristic for different plant families (Asteraceae and Rosaceae)

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EFFECT OF CERTAIN PHENOXY HERBICIDES ON MORTALITY, GROWTH AND SEED OUTPUT OF ABUTILON INDICUM (L.) SW.

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The effect of five phenoxy herbicides viz. MCPA (4-chloro-2-methylphenoxyacetic acid), 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), 2,4,5-TP /2-(2,4,5-tricholorphenoxy)-propionic acid/ and 2,4-DB /4-(2,4-dichlorophenoxy)-butyric acid/ on mortality, growth and seed output of 45-day old plants of Abutilon indicum (L.) Sw. were studied. All the plants died at and above 100 ppm and 1000 ppm concentration when treated aerially with MCPA, 2,4-D and 2,4-DB in one hand and 2,4,5-T and 2,4,5-TP on the other hand, respectively. All the treated plants showed epinastic bending, chlorosis and wilting of leaves, stem splitting and growth arrest. Fasciation and connation of leaves were seen with MCPA, 2,4-D and 2,4-DB. The plants evading death from all applications not only regained vigour in varying length of time according to the type and concentration of herbicides but also had experienced promoted growth and escalated seed output although flowering was delayed by some of the higher concentrations. As such the survivors, of any negligible number may it be, should be in any way killed or else it would beget a serious weed problem through a boosted reproductive potential.

Introduction

Reports of stimulation of plant growth from sublethal doses of herbicides have appeared in literature in conformity with the hypothesis that all poisons are stimulatory in small quantities which was first introduced by SCHULZ (1888) and modified to become the 'Arndt--Schulz rule' (LUCKEY 1959). Stimulatory action of auxin-like phenoxy herbicides on germination is relatively less known (MUKHERJEE 1984b). However, a large number of reports have been received of increased growth and yield of various crops in response to herbicides (RIRIE et al. 1962; PAYNE et al. 1952; ARLE 1954; WORT 1966; LEONARD 1958; HUFFAKER et al. 1967; WIEDMAN and APPLEBY 1972; FAWCETT and SLIFE 1978). Information about the vigour and reproductive potential of weeds that escape death from herbicidal application or experience sublethal concentration of herbicides is so meagre that the present work was under-

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taken. Abutilon indicum (L.) Sw., hairy perennial undershrub with golden yellow flowers and abundance in the hotter parts of India, was selected for the purpose. The findings of this investigation would prove useful in monitoring and alleviating weed problems.

Material and Methods

The herbicides used in this study were MCPA (4-chloro-2-methyl phenoxyacetic acid), 2,4,-D (2,4-dichlorophenoxy acetic acid), 2,4,5-T (2,4,5-tricholorphenoxy acetic acid), 2,4,5-TP /2-(2,4,6-tricholorophenoxy)-propionic acid/ and 2,4-DB /4-(2,4-dichlorophenoxy)-butyric acid/. Aqueous solutions of these were made to give 1, 10, 100, 500 and 1000 ppm (mg/litre) of the active ingredient. The pH of the solutions were adjusted to 7.0.

Healthy seeds were acid scarified (conc. H_2SO_4 for 2 h), washed thoroughly in water and sown in potted soil. When the plants were 45-day-old 10 healthy individuals were selected per pot (45 cm diam) and sprayed with 25 ml of herbicides up to dipping condition of leaves. For each treatment there were four replicates. Following treatment, the percentage of mortality was determined. Growth in terms of shoot height (cm) of treated plants along with those of control plants was recorded when the plants were 300-day-old. At the same time the number of branches and lengths of the longest branch were noted. Furthermore, the time of flowering, fruit and seed output, germinability of seeds were observed. Plants were raised from seeds of treated plants to see whether the toxic effects were transmitted to the next generation or not.

Results

The herbicides MCPA, 2,4-D and 2,4-DB were totally lethal at and above 100 ppm concentration (Table 1). Some mortality was also registered by them at 10 ppm. Both 2,4,5-T and 2,4,5-TP were similar in action, killing most of the plants with 500 ppm and all with 1000 ppm, although at 100 ppm the former was more detrimental. Relevant observations in respect of the phenoxys used is as follows:

MCPA — Plants treated with 100, 500 and 1000 ppm were killed in 31, 18 and 11 days, respectively. With the use of 10—1000 ppm, there was epinastic bending within 24 h. Subsequent yellowing and wilting of leaves as well as swelling in the apical region of the stem with growth arrest became more prominent with increasing concentration of the herbicide. Plants treated with 10 ppm managed to recover not before a month. During this period the plants failed to produce normal leaves.

2,4-D — Plants receiving 100, 500 and 1000 ppm of this herbicide succumbed in 26, 11 and 7 days, respectively. Some of the plants treated with 10 ppm were also killed in about 30 days. With the use of 10—500 ppm the plants showed epinasty within 24 h which was followed by gradual yel-

Table 1

Mortality of Abutilon indicum as affected by aerial spray with herbicides

Herbicides	Concentration (ppm)					
	0 (Control)	1.0	10	100	500	1000
MCPA	7.5 ± 2.5	5.0 ± 5.0	12.5 ± 6.29	100.0 ± 0	100.0 ± 0	100.0 ± 0
2,4-D	5.0 ± 2.9	5.0 ± 2.89	25.0 ± 6.45	100.0 ± 0	100.0 ± 0	100.0 ± 0
2,4,5-T	2.5 ± 2.5	5.0 ± 2.89	12.5 ± 2.50	67.5 ± 4.79	95.0 ± 2.89	100.0 ± 0
2,4,5-TP	0 ± 0	5.0 ± 2.89	5.0 ± 2.89	12.5 ± 2.50	97.5 ± 2.50	100.0 ± 0
2,4-DB	10.0 ± 0	7.5 ± 0	20.0 ± 4.08	100.0 ± 0	100.0 ± 0	100.0 ± 0

lowing and wilting of leaves and growth inhibition. At 1 and 10 ppm the plants showed malformation of leaves in general and connation in particular. The abnormal leaves were thicker, more closely veined and folded with the size and shape totally altered. However, normal leaf formation resumed after 20 and 45 days at 1 and 10 ppm, respectively.

2,4,5-T — All plants sprayed with 1000 ppm were killed in 15 days. Only a few plants survived from treatment with 500 ppm. With the use of 10 to 1000 ppm the plants showed stem swelling, epinasty and wilting; there being no malformation of leaves.

2,4,5-TP — All and most of the plants were killed with 1000 and 500 ppm of this herbicide respectively within 19 days after treatment. At other concentrations mortality was not significant. With the use of 100 and 500 ppm the plants exhibited epinastic curvature within a day with concomittant chlorosis and wilting of leaves and swelling of apical region of stem with growth arrest. Though these treated plants showed recovery in about 40 days they carried swollen stem till flowering.

2,4-DB — Plants sprayed with 100, 500 and 1000 ppm of this herbicide were killed within 30, 10 and 7 days, respectively. These showed epinastic bending, chlorosis and wilting of leaves. Malformation and connation of leaves occurred at 10 ppm. Normal leaf formation resumed after 40 days.

The plants evading death from herbicidal treatment regained vigour so as to have vegetative growth of distinction. These plants revealed, in comparison with untreated ones, promotion of plant height at 1 ppm in case of MCPA, 2,4-D and 2,4-DB and at 1 and 10 ppm in case of 2,4,5-T and 2,4,5-TP (Table 2). A progressive increase in number of branches and length of longest branch were noted in case of all treatments. In this regard 2,4-D,

Table 2

Vegetative growth and flowering of Abutilon indicum plants as affected by aerial spray with herbicides

Herbicide (ppm)	Height of plants (cm)	Number of branches/ plant	Length of the longest branch (cm)	Days after which flowered
MCPA:				
0.0	101.5 ± 5.87	33.6 ± 2.84	26.0 ± 1.39	95
1.0	157.0 ± 5.93	60.3 ± 2.86	45.4 ± 3.26	97
10.0	110.1 ± 9.26	97.2 ± 7.81	63.9 ± 4.21	136
100.0	-	-	-	-
500.0	-	-	-	-
1000.0	-	-	-	-
2,4-D:				
0.0	86.4 ± 2.60	30.2 ± 2.56	22.6 ± 1.45	60
1.0	146.6 ± 3.73	62.2 ± 4.24	64.4 ± 3.59	78
10.0	84.7 ± 3.32	109.5 ± 8.90	77.0 ± 4.35	216
100.0	-	-	-	-
500.0	-	-	-	-
1000.0	-	-	-	-
2,4,5-T:				
0.0	89.6 ± 6.67	38.5 ± 2.16	23.9 ± 1.43	80
1.0	116.5 ± 6.65	38.0 ± 2.38	26.0 ± 1.77	87
10.0	113.6 ± 5.49	98.6 ± 3.89	97.4 ± 5.72	97
100.0	78.8 ± 3.49	107.7 ± 4,74	100.2 ± 5.57	207
500.0	61.5 ± 3.50	91.5 ± 4.51	95.0 ±15.00	210
1000.0	-	-	-	-
2,4,5-TP:				
0.0	103.9 ± 4.31	43.9 ± 2.42	22.0 ± 2.53	79
1.0	140.0 ± 5.01	54.4 ± 4.16	32.1 ± 2.01	72
10.0	144.9 ± 4.52	94.1 ± 5.16	43.2 ± 2.18	75
100.0	108.8 ± 6.23	87.6 ± 4.94	57.6 ± 4.04	97
500.0	*82.0	*76.0	*52.0	130
1000.0	-	-	-	-
2,4-DB:				
0.0	106.3 ± 5.07	34.0 ± 1.58	51.2 ± 1.82	78
1.0	128.3 ± 7.40	51.1 ± 3.46	60.0 ± 3.20	74
10.0	115.2 ± 6.06	70.2 ± 3.75	104.4 ± 7.94	96
100.0	-	-	-	-
500.0	-	-	-	-
1000.0	-	-	-	-

*Readings from single plant were recorded

2,4-DB and MCPA in one hand and 2,4,5-T and 2,4,5-TP on the other hand were similar in action.

Flowering was delayed considerably by MCPA and 2,4-D and slightly by 2,4-DB at 10 ppm (Table 2). In case of 2,4,5-T and 2,4,5-TP flowering was retarded at 100 and 500 ppm, the latter concentration being more effective.

Table 3

Yield of fruits and seeds from Abutilon indicum plants and their germinability as affected by aerial spray with herbicides

Herbicides (ppm)	Fruits/plant	No. of seeds/fruit	Seed output	Germination of seed	Development of seedlings
MCPA:					
0.0	16.1 ± 1.65	39.0 ± 1.61	637.56	62.0 ± 4.50	Normal
1.0	24.3 ± 2.51	43.4 ± 1.39	1054.62	55.7 ± 2.93	Normal
10.0	26.5 ± 2.08	39.8 ± 1.24	1041.45	54.2 ± 2.39	Normal
100.0	-	-	-	-	-
500.0	-	-	-	-	-
1000.0	-	-	-	-	-
2,4-D:					
0.0	17.2 ± 1.65	30.8 ± 1.09	529.76	72.2 ± 3.61	Normal
1.0	29.0 ± 2.30	35.3 ± 1.17	1023.70	68.7 ± 5.65	Normal
10.0	33.0 ± 2.48	35.8 ± 1.14	1181.40	75.5 ± 6.61	Normal
100.0	-	-	-	-	-
500.0	-	-	-	-	-
1000.0	-	-	-	-	-
2,4,5-T:					
0.0	17.0 ± 1.54	30.8 ± 1.09	680.03	64.0 ± 5.75	Normal
1.0	26.5 ± 2.12	41.9 ± 1.00	1110.35	70.2 ± 4.61	Normal
10.0	24.0 ± 2.29	39.1 ± 1.20	938.40	71.5 ± 4.27	Normal
100.0	25.2 ± 1.77	42.2 ± 3.56	1063.44	69.2 ± 8.11	Normal
500.0	24.0 ± 4.54	43.2 ± 1.13	1036.80	70.7 ± 4.77	Normal
1000.0	-	-	-	-	-
2,4,5-TP:					
0.0	19.6 ± 0.96	36.8 ± 1.88	721.28	65.2 ± 2.93	Normal
1.0	23.8 ± 1.90	38.4 ± 1.50	913.92	61.2 ± 3.54	Normal
10.0	28.0 ± 1.75	38.4 ± 1.30	1075.20	64.0 ± 4.97	Normal
100.0	29.3 ± 1.21	38.2 ± 1.61	1060.66	69.2 ± 4.23	Normal
500.0	27.0*	37.0 ± 3.12	999.00	72.5 ± 4.11	Normal
1000.0	-	-	-	-	-
2,4-DB:					
0.0	20.0 ± 1.64	39.0 ± 1.98	788.00	76.5 ± 6.14	Normal
1.0	26.2 ± 1.53	39.5 ± 2.83	1034.90	70.5 ± 5.74	Normal
10.0	27.8 ± 1.74	39.8 ± 1.26	1045.20	73.2 ± 4.19	Normal
100.0	-	-	-	-	-
500.0	-	-	-	-	-
1000.0	-	-	-	-	-

*Reading from single plant was recorded.

There was an increase in the yield of fruits and seeds at 1 and 10 ppm of 2,4-D, MCPA and 2,4-DB. More number of fruits and seeds were obtained with all treatments (1--500 ppm) of 2,4,5-T and 2,4,5-TP as compared to those of control. Seeds obtained from all the treated plants germinated normally and produced normal seedlings.

Discussion

MCPA, 2,4-D and 2,4-DB are more severe than 2,4,5-T and 2,4,5-TP in extending detriment to the 45-day-old plants. These phenoxy compounds, although have a differential action, may involve common avenues to establish similar effects. Excessive nucleic acid and protein synthesis induced by 2,4-D and relatives could interfere with normal development and function (SHANNON et al. 1964; MUKHERJEE 1990). Lower and sublethal concentrations of phenoxys probably rearrange the endogenous hormonal leves to such an extent that plant growth and reproductive potential are increased. RIES (1976) has made a valuable review of the subtoxic effect of herbicides on plants to provide information about biochemical basis for the stimulatory effect of subtoxic levels of herbidies. Higher dosages of phenoxys probably compete with endogenous hormones to exaggerate and derange metabolic and physical processes so as to kill the plant. Epinastic curvature is accompanied by other symptoms of toxicity like chlorosis, swelling, stem splitting and growth arrest leading to death (BEAL 1944; HAMNER and TUKEY 1946; RODGERS 1952; SAHA 1972; MARTIN and FLETCHER 1972; MITRA and SINGH 1972; BAKALE and HADKE 1978; BAKALE and KOLHE 1978; MUKHERJEE and DATTA 1980). Spectacular changes in leaf structure as induced by 2,4-D, 2,4-DB and MCPA in Abutilon indicum were also seen by GIFFORD (1953) in cotton plant following 2,4-D administration. Fasciation and connation of leaves resulted possibly from disturbance in correlation wrought by these herbicides. GORTER and VAN DER ZWEEP (1964) as well as VAN ANDEL et al. (1976) have looked for the morphogenetic changes in plants under herbicidal impact.

The aforesaid abnormalities induced by sublethal dosages could be overcome by plants in varying lengths of time according to concentration and type of herbicide used. The progressive increase in number of branches per plant and length of the longest branch with increase in concentration of all phenoxys was presumably due to partial or total loss of apical domiance, the apex being killed or seriously damaged and acceleration of the formation of axillary branches. Similar effect was seen in Arachis hypogea, Carthamus tinctorius and Linum usitatissimum by SINGH and SINGH (1975).

The study of the action of herbicides on reproductive attributes of plants has received less attention (MUKHERJEE 1984a). This is not surprising because the eradication os weeds is emphasized either before emergence (DE DATTA 1972) or at an early and active stage of growth before flowering (BOTTON 1973; LINK 1976). Sublethal concentration of phenoxys delay flower-

ing. Probably this retardation does not involve the so-called florigen complex and is possibly and indirect effect mediated through inhibition of plant growth. Despite delayed flowering, these plants possibly undergo certain metabolis rearrangement leading to increased reproductive potential manifested in form of fruit and seed yield. The seeds, thus obtained, were all normal in form and function. 2,4-D was the most successful herbicide in this respect where there were 57.5%, 68.6% and 91.9% increase with 0.1, 1.0 and 10 ppm, respectively. MCPA was less active than 2,4-D and the enhancement caused by other herbicides at different concentrations hardly exceeded 50%.

The examination of seed viability and seedling behaviour have in no case revealed any sign of persistence and propagation of the toxic effect of herbicides to the next generation. These herbicides failed to reduce reproductive structure and yield and to transmit its effects to the next generation probably because the plants were very young when they were treated and there was a long interval between the time of treatment and the appearance of flowers and fruits. Furthermore these do not act as mutagens. A similar explanation was received from RODGERS et al. (1952) and MITRA and SINGH (1972).

From the foregoing it is evident that the herbicides, when failure to kill all weeds in any crop field or elsewhere can escalate the weed problem by giving and additional boost to their reproductive potential. In any weed eradication programme proper selection of the herbicide, dosage and time is necessary since the herbicides are highly selective. With every attempt to control weeds with phenoxy herbicides there should be a close watch on the weed mortality. Any survivor, of any negligible number may it be, should be killed or removed or else it would again disperse to the verge of appreciable obnoxiousness by its enhanced seed output.

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STRUCTURE AND DISTRIBUTION OF MUCILAGE CELLS IN LEAF EPIDERMIS OF MALVALES

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Morphology and distribution of Mucilagenous cells occurring in leaf epidermis of fifty species of Malvales are described. Thirty-four species were found to possess Mucilagenous cells. The leaves are described as amphi-, hypo- and epimucilagenous. Based on the distribution of the Mucilagenous cells on a given surface of the leaf, in all six distributional patterns are described. The taxonomic significance of these cells are discussed.

Introduction

Occurrence of mucilage cells in plant tissue is considered as an important criterion in the field of systematic anatomy (SOLEREDER 1908; METCALFE and CHALK 1950, 1983). Mucilagenous cells in mesophyll and ground tissue is a characteristic feature of Malvales (SOLEREDER 1908; METCALFE and CHALK 1950, 1983). But they have been rarely reported in the leaf epidermis (GREGORY and BASS 1989): Further the earlier records on the mucilagenous cells in the epidermis do not report the differential distribution of these cells on a given surface of a leaf, which could be taxonomically valuable. Hence the present investigation is taken up to study in detail the morphology, distribution and taxonomic significance of mucilagenous cells in the epidermis of Malvales.

Material and Methods

Mature leaves of 50 species of Malvales, collected from different places of India were fixed in Carnoy's fixative (JOHANSEN 1940). Ten epidermal peels of different leaves of each species collected from five plants were prepared from base to apex and midvein to margins of the leaf following the procedure of LEELAVATHI and RAMAYYA (1975). Peels were stained with methylen green/crystal voilet/basic fuchsin/safranin and mounted in glycerine.

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Observations and Discussion

Presently the mucilagenous cells are recorded in 19 genera and 34 species of Malvales (Table 1) but absent in sixteen Abutilon crispum (L.) Medic., A. glaucum (Cav.) Sweet, Bombax malabaricum DC., Eriodendron pentandrum Kurz., Gossypium arboreum L., G. herbaceum L., Hibiscus tiliaceus L., Thespesia populnea (L.) sol. ex. corr., Sida rhombifolia L., Helicteres isora L., Melhania incana Heyne ex W. & A., Pterospermum acerifolium Willd., Corchorus acutangulus Lamk., C. olitorius L., Muntingia calabura L., Triumfetta rhomboidea Jacq. Contrary to the earlier observations (INAMDAR and CHOHAN 1969a, b; RAXO and RAMAYYA 1984), the mucilagenous cells are recorded in epidermis of Althaea rosea, Buettneria, Guazuma, Hibiscus cannabinus, H. rosa-sinensis, Melochia corchorifolia, Sida acuta, S. cordata, S. cardifolia, S. glutinosa, S. spinosa, Sterculia foetida, Thespesia lampas, Triumfetta pentandra and Waltheria indica (Figs 1C, D, I; 2. A--D, G--I, K; Table 1). Further in Bombax malabaricum and Eriodendron pentandrum the mucilagenous cells were reported to be present (RAO and RAMAYYA 1984), but in the present study they were found to be absent (Figs 1A, B; Table 1).

The leaves are presently described as amphimucilagenous (mucilagenous cells present in both adaxial and abaxial epidermis), epimucilagenous or hypomucilagenous (mucilagenous cells either present in adaxial/abaxial epidermis). The leaves of Althaea rosea, Buettneria herbacea and 21 other taxa are amphimucilagenous. In Dombeya cayeuxii and other nine taxa they are epimucilagenous and those of Thespesia lampas are hypomucilagenous (Table 2).

The mucilagenous cells are similar to the epidermal cell in shape, size and anticlinal walls as in Sida, Buettneria, Dombeya, Guazuma, Kleinhovia, Sterculia, Corchorus and Triumfetta (Figs 2A--C), while in Hibiscus lobatus, H. micranthus, H. rosa-sinensis, Althaea, Malvastrum, Malvaviscus, Pavonia and Melochia, some of the mucilagenous cells are distinctly small in size and variable in shape being circular, oval, squarish, rectangular or reniform, while others similar to epidermal cells (Figs 2A--C). In Adansonia, Hibiscus cannabinus, Grewia, Thespesia and Waltheria all the mucilagenous cells are either distinctly smaller or larger in size and also vary in shape from the other epidermal cells (Figs 2F, G; Table 2).

Histochemically it was observed that all the mucilagenous cells in a given taxon react positively or negatively with a particular stain, while in others some of the mucilagenous cells react positively while others react negatively. For example the mucilagenous cells on abaxial and adaxial sur-

Table 1

Distribution of mucilagenous cells in epidermis of Malvales

	Eip Ad/Ab	Sub Ad/Ab	Gc Ad/Ab	Mv Ad/Ab	Sv Ad/Ab	Tv Ad/Ab
MALVACEAE						
1. Abelmoschus ficulneus (L.) W. & A. ex. W.	+/+	-/-	-/-	-/-	-/-	-/-
2. Adansonia digitata L.	+/+	-/-	+/-	-/-	-/-	-/-
3. Althaea rosea Cav.	+/+	-/-	-/-	+/+	+/+	-/-
4. Hibiscus cannabinus L.	+/+	-/-	-/-	-/-	-/-	-/-
5. H. lobatus (Merr.) Kuntze	+/+	-/-	-/-	-/-	-/-	-/-
6. H. micranthus L.	+/+	-/-	-/-	-/-	-/-	-/-
7. H. rosasinensis L.	+/+	-/+	-/-	-/-	-/-	-/-
8. H. sabdariffa L.	+/+	-/-	+/-	-/-	-/-	-/-
9. H. vitifolius L.	+/+	-/-	-/-	-/-	-/-	-/-
10. Malvastrum cormandelianus (L.) Garcke.	+/+	+/+	-/-	+/+	+/+	-/-
11. Malvaviscus arboreus Cav.	+/+	-/-	-/-	-/-	-/-	-/-
12. Pavonia odorata Willd.	+/+	-/-	-/-	-/-	-/-	-/-
13. P. zeylanica (L.) Cav.	+/+	+/-	-/-	-/-	-/-	-/-
14. Thespesia lampas (Cav.) Dalz. ex. Dalz. & Gibs.	-/+	-/-	-/-	-/-	-/+	-/+
15. Sida acuta Burm.	+/+	-/-	-/-	-/-	-/-	-/-
16. S. cordata (N. Burm.) Borss.	+/+	-/-	-/-	-/-	-/-	-/-
17. S. cordifolia L.	+/+	-/-	-/-	-/-	-/-	-/-
18. S. glutinosa Cav.	+/+	-/-	-/-	-/-	-/-	-/-
19. S. spinosa L.	+/+	-/-	-/-	-/-	-/-	-/-
STERCULIACEAE						
20. Buettneria herbacea Roxb.	+/+	-/-	-/-	-/-	-/-	-/-
21. Dombeya cayeuxii Hort.	+/-	-/-	-/-	-/-	-/-	-/-
22. Guazuma tomentosa Kunth.	+/-	-/-	-/-	-/-	-/-	-/-
23. Kleinhovia hospita L.	+/-	-/-	-/-	-/-	-/-	-/-
24. Melochia corchorifolia L.	+/+	+/+	-/-	+/+	+/+	-/-
25. Sterculia foetida L.	+/-	-/-	-/-	-/-	-/-	-/-
26. Waltheria indica L.	+/-	+/-	-/-	-/-	-/-	-/-
TILIACEAE						
27. C. fascicularis Lamk.	+/+	-/-	-/-	-/-	-/-	-/-
28. C. tridens L.	+/+	-/-	-/-	-/-	-/-	-/-
29. C. trilocularis L.	+/+	-/-	-/-	-/-	-/-	-/-
30. C. urticaefolius W. & A.	+/+	-/-	-/-	-/-	-/-	-/-
31. Grewia flavescens Juss.	-/-	-/-	-/-	+/-	-/-	-/-
32. G. hirsuta Vahl.	-/-	-/-	-/-	-/-	+/-	+/-
33. G. tiliaefolia Vahl.	-/-	-/-	-/-	+/-	-/-	-/-
34. Triumfetta pentandra A. Rich.	+/-	-/-	-/-	-/-	-/-	-/-

Ab = abaxial; Ad = adaxial; Eip = epidermis; Gc = guard cell; Mv = midvein; Sub = subsididary cells; Sv = secondary vein; Tv = tertiary vein; + = present; - = absent.

Table 2

Types of leaves and characters of mucilagenous cells in epidermis of Malvales

	Am	H	Ep	Dis	Ind	Sol	Pa	Cl	P	Re	R	S	O	C
MALVACEAE														
1. Abelmoschus ficulneus	+	-	-	+	+	-	+	+	+	-	-	-	+	+
2. Adansonia digitata	+	-	-	+	-	+	-	-	-	-	-	-	-	+
3. Althaea rosea	+	-	-	+	+	+	+	+	+	-	-	-	+	+
4. Hibiscus cannabinus	+	-	-	+	-	+	-	-	+	-	-	+	-	+
5. H. lobatus	+	-	-	+	+	-	+	+	+	+	-	-	-	+
6. H. micranthus	+	-	-	+	+	-	+	+	+	-	-	-	+	+
7. H. rosa-sinensis	+	-	-	+	+	+	-	-	-	-	+	-	-	+
8. H. sabdariffa	+	-	-	-	+	-	+	+	+	-	-	+	+	+
9. H. vitifolius	+	-	-	-	+	+	-	-	+	-	-	-	+	+
10. Malvastrum cormandelianum	+	-	-	-	+	-	+	+	+	-	+	-	+	+
11. Malvaviscus arboreus	+	-	-	+	+	-	+	+	-	-	+	+	+	+
12. Pavonia odorata	+	-	-	+	+	+	+	+	+	-	-	+	+	+
13. P. zeylanica	+	-	-	+	+	+	+	+	-	+	-	-	-	-
14. Thespesia lampas	-	+	-	+	-	+	+	+	-	-	-	-	-	-
15. Sida acuta	+	-	-	-	+	+	+	-	+	-	-	-	-	-
16. S. cordata	+	-	-	-	+	+	+	-	+	-	-	-	-	-
17. S. cordifolia	+	-	-	-	+	+	-	-	+	-	-	-	-	-
18. S. glutinosa	+	-	-	-	+	+	+	+	+	-	-	-	-	-
19. S. spinosa	+	-	-	-	+	+	+	+	+	-	-	-	-	-
STERCULIACEAE														
20. Buettneria herbacea	+	-	-	-	+	+	+	+	-	-	+	+	-	-
21. Dombeya cayeuxii	-	-	+	-	+	-	+	+	+	-	-	-	-	-
22. Guazuma tomentosa	-	-	+	-	+	+	-	-	+	-	-	-	-	-
23. Kleinhovia hospita	-	-	+	-	+	+	-	-	+	-	-	-	-	-
24. Melochia corchorifolia	+	-	-	+	+	+	+	+	+	-	+	-	-	-
25. Sterculia foetida	-	-	+	-	+	+	-	-	+	-	-	-	-	-
26. Waltheria indica	-	-	+	+	-	+	+	-	+	+	-	-	-	+
TILIACEAE														
27. C. fascicularis	+	-	-	-	+	+	+	-	+	-	-	-	-	-
28. C. tridens	+	-	-	+	+	+	+	+	+	+	+	-	+	+
29. C. trilocularis	-	-	+	-	+	+	+	-	+	-	-	-	-	-
30. C. urticaefolius	+	-	-	-	+	+	+	-	+	-	-	-	-	-
31. Grewia flavescens	-	-	+	+	-	-	+	-	-	-	-	-	-	+
32. G. hirsuta	-	-	+	+	-	+	+	-	-	-	-	+	-	+
33. G. tiliaefolia	-	-	+	+	-	+	-	-	-	-	-	+	-	+
34. Triumfetta pentandra	-	-	+	-	+	+	-	-	+	-	-	-	-	-

Am = amphimucilagenous; C = circular; Cl = cluster; Dis = distinct; Ep = epimucilagenous; H = hypomucilagenous; Ind = indistinct; O = oval; P = polygonal; Pa = pair; R = rectangular; Re = reniform; S = squarish; Sol = solitary; + = present; - = absent.

faces of Sida acuta react positively with safranin (Fig. 2A), while negatively in Sterculia foetida (Fig. 2H). In Hibiscus lobatus some of the mucilagenous cell react positively, while others negatively with basic fachsin (Fig. 1E), probably indicating the acidic or neutral nature of the mucilage. Some of the mucilagenous cells were also found to contain calcium oxalate crystals (Fig. 1I). Very high intensity of light is required to visualise these crystals either under polarized or ordinary light microscope. The mucilagenous cells are either solitary interspersed with the other epidermal cells as in Triumfetta pentandra and nine other taxa (Figs 2K; Table 2) or solitary and also in pairs as in Abelmoschus ficulneus and seven other taxa (Fig. 2E). While they are in pairs and also in isolated clusters (of 2-4 cells) in Malvaviscus arboreus and six other taxa and in Althaea rosea and eight other taxa they are solitary, in pairs as well as in clusters (Figs 2G, I; Table 2).

The mucilagenous cells have been reported to be absent in the costal zones (RAO and RAMAYYA 1984). Presently they have been observed only in costal zone of Grewia (Table 1) or distributed both in the intercostal and costal zones as in Althaea rosea, Malvastrum coromandelianum, Thespesia lampas and Melochia corchorifolia (Figs 1J, K). While in rest of the 27 taxa they are restricted to intercostal areas (Table 1). Among the intercostal cells (in some taxa), the mucilage may be present only in epidermal cells or epidermal cells and subsidary cells (Fig. 2I) and or guard cells (Fig. 1G) which is of further taxonomic significance (Table 1).

On the basis of comparative study of the mucilagenous cell's distribution, the following six distributional patterns are recorded:

Pattern 1. Mucilage present only in intercostal epidermal cells as in adaxial and abaxial of Hibiscus lobatus and other 21 taxa (Figs 2J, K; Table 1).

Pattern 2. Mucilage present only in adaxial costal cells (as in Grewia flavescens, G. hirsuta and G. tiliaefolia (Fig. 1K; Table 1).

Pattern 3. Mucilage present in intercostal epidermal and costal cells as in adaxial and abaxial of Althaea rosea, Malvastrum coromadelianum and abaxial of Thespesia lampas (Figs 1J, K; Table 1).

Pattern 4. Mucilage present in intercostal epidermal cells and subsidiary cells as in adaxial and abaxial of Hibiscus rosa-sinensis, Pavonia zeylanica and Waltheria indica (Figs 1H, 2I; Table 1).

Pattern 5. Mucilage present in intercostal epidermal cells and guard cells as in adaxial and abaxial of Adansonia digitata and Hibiscus sabdariffa (Fig. 2G; Table 1).
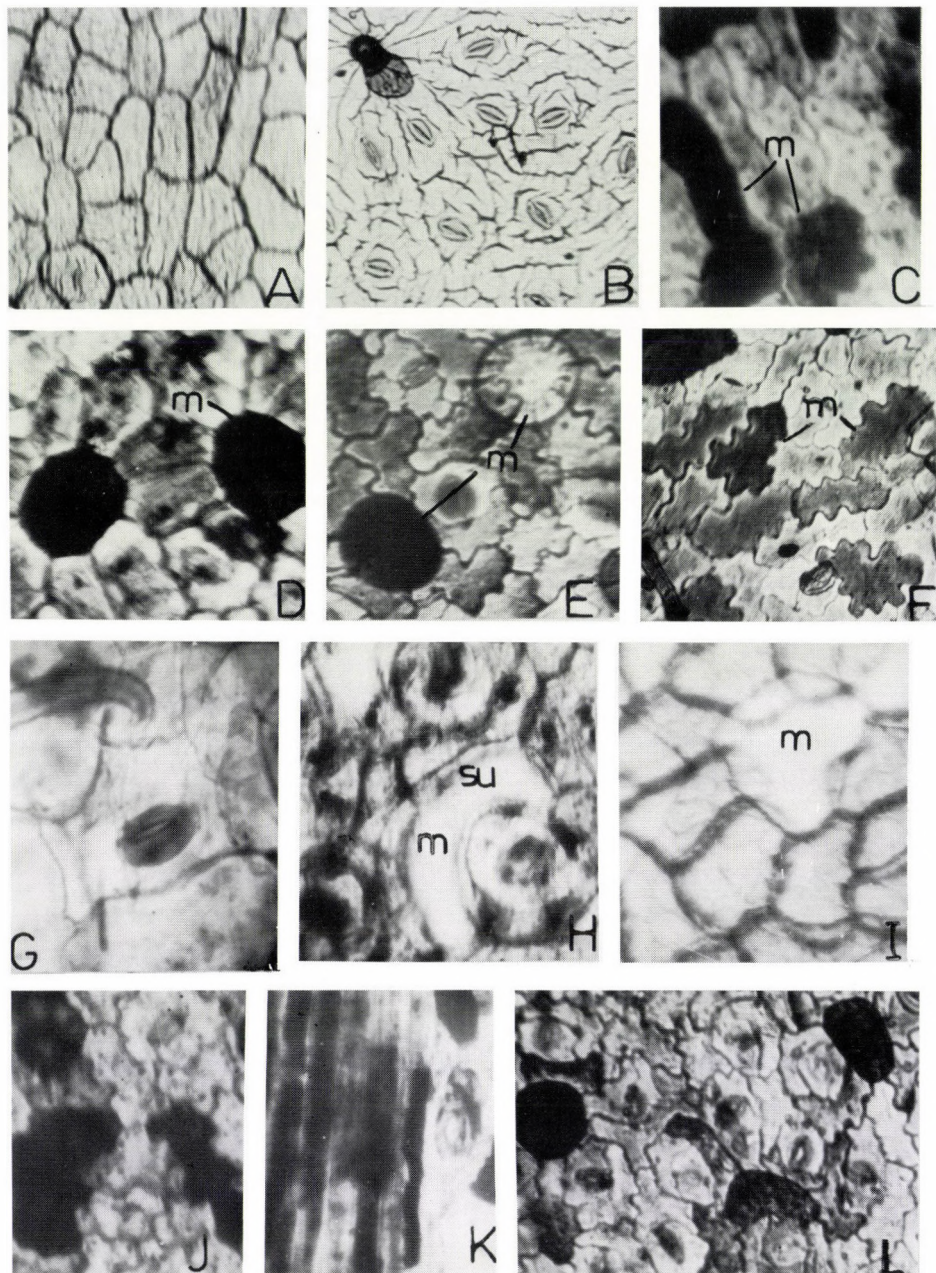
Fig. 1A—B. (x 145) Eriodendron pentandrum adaxial and abaxial epidermis lacking mucilagenous cells. C—J, L. (x 145). Epidermis showing mucilagenous cells in intercostal area. C. Althaea rosea adaxial, D. Hibiscus cannabinus adaxial (mucilagenous cells solitary), E, F. Hibiscus lobatus adaxial and abaxial respectively (mucilagenous cells in pairs and clusters), G. Hibiscus sabdariffa adaxial (mucilage in guard cells), H, I. Hibiscus rosa-sinensis adaxial and abaxial, respectively (mucilage in subsidiaries), J, K. Malvastrum coromandelianum adaxial (intercostal zones) and abaxial (x 1000) costal zone), respectively. L. Pavonia zeylanica adaxial (m = mucilage, su = subsidiaries)
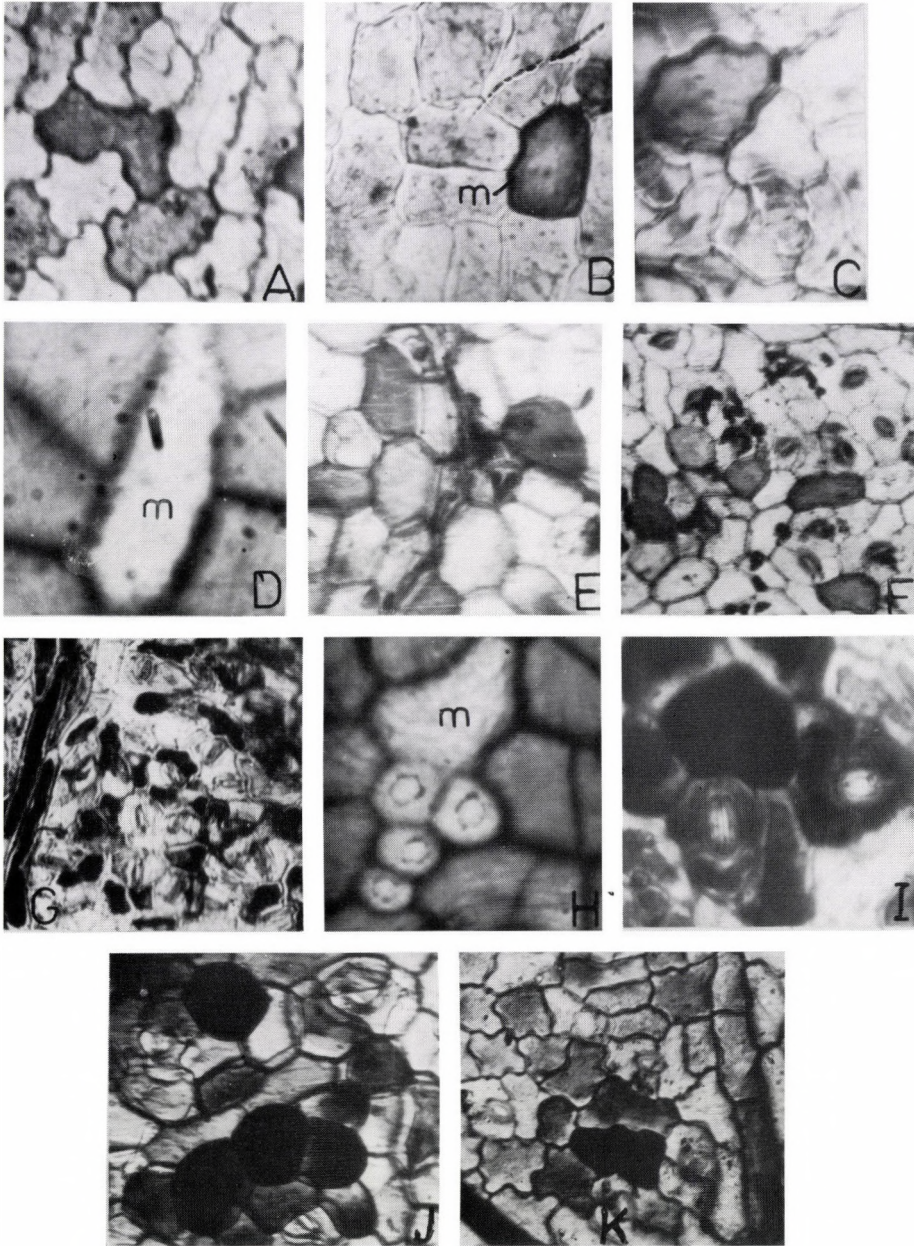
Fig. 2A—D, H, I (x 1000), E—G, J, K (x 145). Epidermis showing mucilagenous cells in intercostal areas. A. Sida acuta adaxial (mucilage cells in pairs), B. S. cordata adaxial, C. S. cordifolia adaxial, D. Guazuma tomentosa adaxial (mucilage cells solitary), E, F. Buettneria herbecea adaxial and abaxial, respectively, G. Melochia corchorifolia adaxial epidermis showing mucilage cells in costal and intercostal zones, H. Sterculia foetida adaxial, I. Waltheria indica adaxial, J. Corchorus tridens adaxial (mucilage cells in clusters), K. Triumfetta pentandra adaxial (m = mucilagenous cells)

Pattern 6. Mucilage present in intercostal epidermal cells, costal and subsidary cells as in adaxial and abaxial of Melochia corchorifolia (Figs 1J, L; 2G; Table 1).

The genus Grewia is taxonomically distinct in having mucilagenous cells only in costal areas while in the genera Adansonia, Hibiscus, Malvaviscus, Pavonia, Sida (Malvaceae); Buettneria, Dombeya, Guazuma, Kleinhovia, Sterculia, Waltheria (Sterculiaceae); Corchorus and Triumfetta (Tiliaceae), they are restricted to intercostal areas but in Althaea, Malvastrum, Thespesia (Malvaceae) and Melochia (Sterculiaceae), they are present both in intercostal as well as costal areas. The characteristics of the mucilagenous cells in conjunction with other epidermal characters could be more taxonomically significant.

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SEM ANALYSIS ON THE LEAF EPIDERMIS OF THE CUBAN CLERODENDRUM TAXA (VERBENACEAE)

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A comparative scanning electron microscopical study on the epidermis taken from 54 herbarium specimens of the Cuban mostly endemic Clerodendrum species revealed species—specific epidermal patterns in most species. The characteristics appear to be of particular value in a more exact taxonomic delimiation of the species, above all at infraspecific level. The examination of most typical 54 specimens in the Herbarium BP, HAC, HAJB, KW and S provides distinct epidermal features to separate new infraspecific taxa in C. aculeatum, C. grandiflorum and C. cubense. Recognition some characteristic features of stoma and hair structure has supported the macromorphologic evaluation of the new taxa as well (KERESZTY 1991). The previous hypothesis that C. calcicola and C. tuberculatum have to be conspecific, is conspicuously reinforced by means of micrographs. The revealed different stoma-types correlating with their ecological conditions indicate relationships between the taxa studied may permit to design a possible phylogenetic descent of the species as well.

Introduction

Since previous classification of Cuban Clerodendrum species was based mainly on external morphological characteristics of herbarium specimens, new approaches using up to data techniques were necessary to understand better the systematics of these plants. The aim of this study was to evaluate the anatomical elements of the leaf epidermis by scanning electron microscope (SEM) and use them as additional criteria for the delimiation of taxa. The morphology of the leaves of Clerodendrum was examined by GRISEBACH (1866) in Göttingen on the WRIGHT's, or URBAN (1911) in Berlin—Dahlem on the EKMAN's collections. The achieved results were summarized by H. LEÓN and H. ALAIN in the 5 volumes of the Flora de Cuba (1946—1962). According this work represented in Cuba by 9 endemic and several cultivated species. MOLDENKE indicated the high taxonomic significance of leaf surface charac-

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teristics (1985—87) examined with light microscopy.[1] SEM analysis on the representatives of Clerodendrum leaves were made first by the author of this article. The strong discriminative value of the special features of the lower surface and in particular of the stomatal cells were demonstrated in this way.

The preparation of the new Flora de Cuba makes necessary to revise the latest collections and the present grouping of the genus. The comparative study based on micrographs of 54 specimens of the herbaria BP, HAC, HAJB, KW and S revealed new elements for the revision of the genus (KERESZTY 1992). The taxonomic importance of the stomatal structure in recognizing new varietates and forms is convincingly proved. Characteristics observed on the micrographs indicate taxonomic relations between taxa and permit tracing some phylogenetic conclusions.

Material and Method

Leaf materials used in this study were obtained from herbarium specimens mostly in HAC and HAJB, partly in BP (BORHIDI et al.), KW (TURCZANINOV) and S (EKMAN) herbariums, where larger, former or recent collections from Cuba are found. Fifty-four following representative specimens were selected from more then 200 ones of 18 taxa prepared with light microscope for the SEM analyses (see: Appendix).

Two leaf segments of 3 x 5 mm cut from the middle part of mature air-dried leaves were coated with gold without any previous treatment, and examined both their upper and lower surfaces with JEOL JSM 35C scanning electron microscope.

Description of the epidermal features

Upper leaf surface

Two tissue-shape on the upper leaf surface of the native species in Cuba are to be distinguished. Epidermis is usually covered by thick, contiguous and variously rolled up layer of cuticle, by that is hidden the cell-structure. The wrinkles of the cuticle of C. nipense is scarcely prominent, mostly straight or waved (Fig. 1), that of C. lindenianum is conspicuously prominent, composed by dense or loose irregular wrinkles (Fig. 2). C. acu-

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[1]The taxonomic importance of the epidermis structure in Verbenaceae has been recently demonstrated by CANTINO (1990).

leatum group and C. grandiflorum group have another shape of the surface. The cell-structure of the epidermis can be seen through the thin cuticle. This is in the case of C. grandiflorum cubiform (Fig. 3), of C. aculeatum irregularly "gravelly" bulged (Fig. 4). There are sometimes densely micro-papilla risen on the cuticle (Fig. 3 arrows). The irregular upper surfaces of C. calcicola (Fig. 6) and C. cubense (Fig. 5) considered as an intermediate form. The cell-structure of the epidermis is to be seen in spots through the thin cuticle. There are scattered, occasionally sunken glands on the surface. Short, conical, partly embedded uni- and multicellular trichomes are to be found occasionally, but they are much more sparsely as on the lower surface (Figs 1, 4).

Lower leaf surface

The cuticle layer is in the case of some species thin (C. aculeatum group) sometimes imitating the cell structure. He is moustly thick, composed by waved wrinkles. This thick cuticle is just partely to remove even by a long-drown preparing methods (ABU-ASAB, CANTINO 1987). Therefore the stoma-system based on subsidiary cells can use unambiguously only in species having thin cuticle layer. In the examination of the prepared slides emphasis was placed on two sets of characters that ABU-ASAB and CANTINO (1987) found to be of systematic significance in the two related families, Lamiaceae and Verbenaceae: the morphology of the stomatal complexes and the structure of the subsessile glandular trichomes. As for CANTINO (1990) anomocytic stomata were the most frequently encountered types in Verbenaceae as well as in the genus Clerodendrum. He investigated two Clerodendrum species native in Cuba: C. aculeatum and C. anafense (1990: 335. Table 1). Anomocytic and actinocytic types were the significant in both species. In C. anafense some anisocytic and paracytic stomate were found as well. By reason of SEM investigations the structure of the cuticle wrinkles around the stomata proved to be more significant than the subsidiary cells, suggesting a potential descending sequence of the species. Stomata have a conspicuous suprastomatal cavity (Fig. 21 arrow) and are risen, lied on the level or variously embedded. The formation of the cuticle around the stomata are considered moustly as a specific character by WILKINSON as well (1979: 107). On the basis of these forms can be separated the following stoma type:

— cubense type: stomata are among the wrinkles or upon them. Cuticle around them does not compose special form. The mean length of the stomata 13 μ (Figs 13—14).

— aculeatum type: regular radial wrinkle-bundles are running down from the prominent stomata provided with parallel microlines, composing a star-shaped formation. The length is varying from 15 to 20 μ (Fig. 7).

— anafense type: stomata raised slightly in a flat pit of the epidermis, surrounded by major irregular and to the stoma-pore perpendicular fine wrinkles, with the length 17 μ (Figs 11—12).

— nipense type: cuticle composes an arc- or half arc-shaped wrinkles around the sunken stomata with very changing length from 13 to 17 μ (Fig. 21).

From the high correlation between the stoma-types and the localities may be concluded, that the taxonomical diversity is connected with the geobotanical distribution. From this fact, taking into account their endemic character, can be conclude to the potential evolutive trends.

In the native Cuban Clerodendrum species there are only hypostomatic leaves.

The lower surface is either completely hairless (Fig. 17) or covered by conical, rigid uni- or multicellular glandular trichomes, sometimes also by different-shaped hairs (Fig. 10). The rigid trichomes have large, enlarged base and many transparent small dots (Fig. 9). There are many glands among them. Since subsessile glandular trichomes are present in nearly all the Verbenaceae -- functioning in the secretion and storage of the essential oils (terpenoids) that characterize the family (BRUNI, MODENESI 1983) -- they may be used as taxonomic characters. ABU-ASAB and CANTINO (1987) developed a classification of 11 subsessile gland types based on the number of cells and the cell-wall configurations in the head of the gland (CANTINO 1990, Fig. 2). The adjective "subsessile" was applied by the authors because the glands may appear sessile in surface view but can be seen in cross section to have a short usually discoid stalk cell (FAHN 1979, Fig. 92). They are generally more common on the abaxial than the adaxial surface of the leaf. Types 4 and 5 are the most frequently within the Verbenaceae as well as in the genus Clerodendrum. They are consisting from 4-6 cells.

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Plate I. Upper leaf surfaces

Figs 1—6. 1. C. nipense var. pubescens; Srra del Cristal, ALAIN et al. 5349, x 400. — 2. C. lindenianum; Srra de Nipe, EKMAN 6736, x 600. — 3. C. grandiflorum; PR, Bahia Honda, LEÓN 20974, x 540. — 4. C. aculeatum var. gracile; Hab. Guanabacoa, MOLDENKE 19859, x 540. — 5. C. cubense; SAGRA 2866, x 200. — 6. C. calcicola; PR; Guanahacabibes, BISSE et al. 30907, x 540
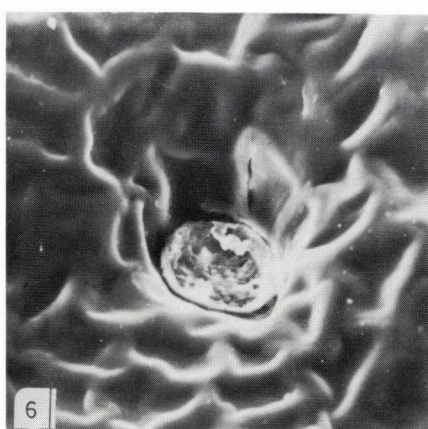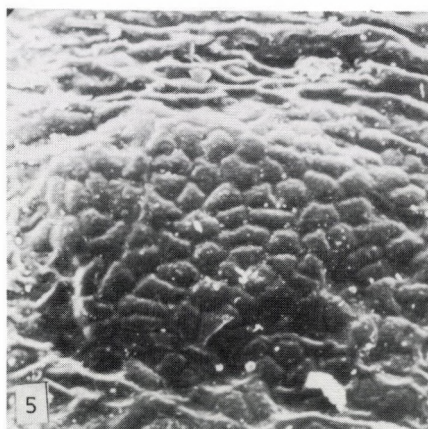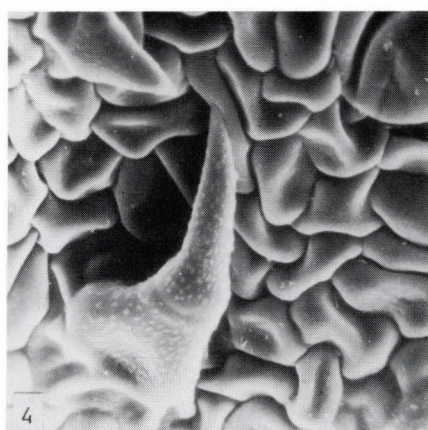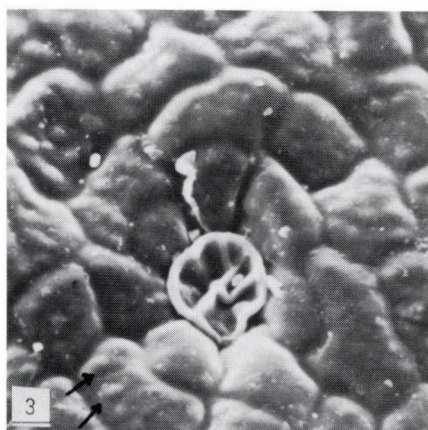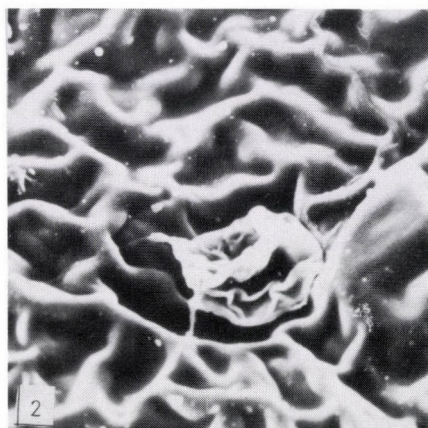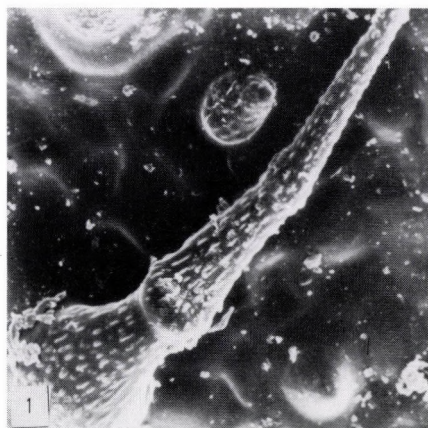
Plate I
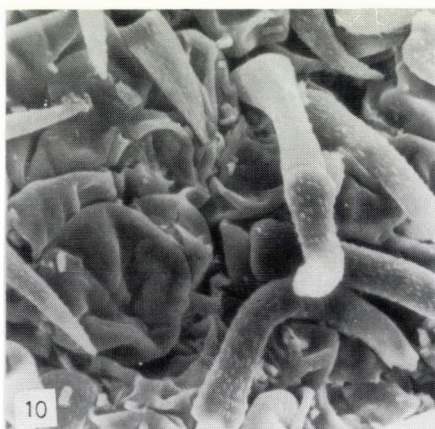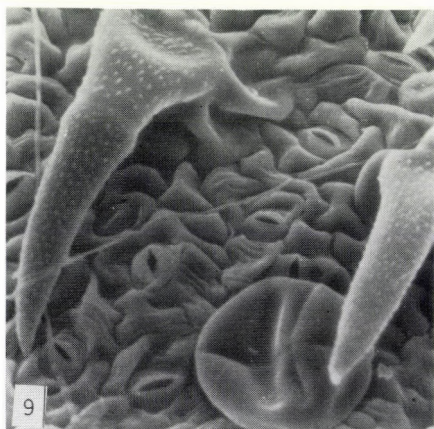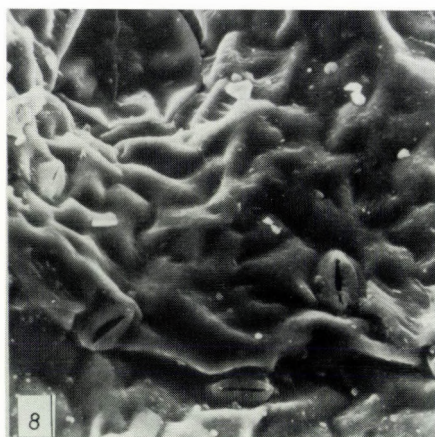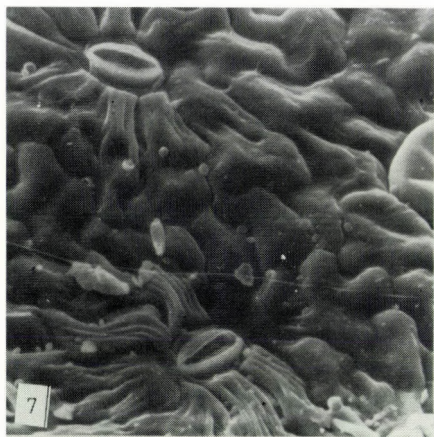
Plate II. Lower leaf surfaces

Figs 7—10. 7. C. aculeatum f. acutifolium; St. Croix, SWARTZ 283, x 540. — 8. C. aculeatum f. rotundatum; St. Bartholomew (Swartz), x 540. — 9. C. aculeatum var. gracile; Hab. Guanabacoa, MOLDENKE 19859, x 540. — 10. C. aculeatum var. gracile f. orientale; Sgo de Cuba, BORHIDI et MUÑIZ 518915, x 540

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Plate III. Lower leaf surfaces

Figs 11—16. 11. C. anafense; Srra de Anafe, WILSON 11466, x 1000. — 12. C. denticulatum; OR, Mog. Baire, BORHIDI et MUÑIZ BP 517718, x 540. — 13. C. cubense; PR. Srra del Rosario, IMCHANITZKAJA 33604, x 400. — 14. C. cubense var. brachypus; HAC (Wright) 3175, x 540. — 15. C. grandiflorum; PR: Bahia Honda, LEÓN 20974, x 540. — 16. C. grandiflorum ssp. cajalbanense; PR. Cajalbana, ACUÑA 16417, x 540
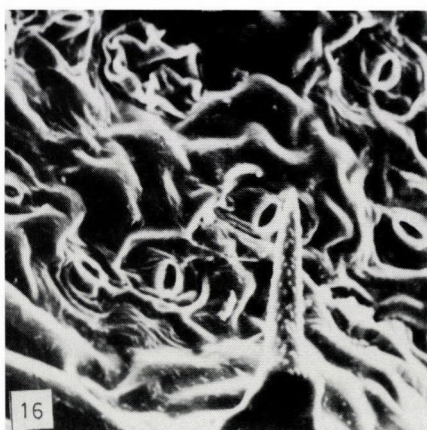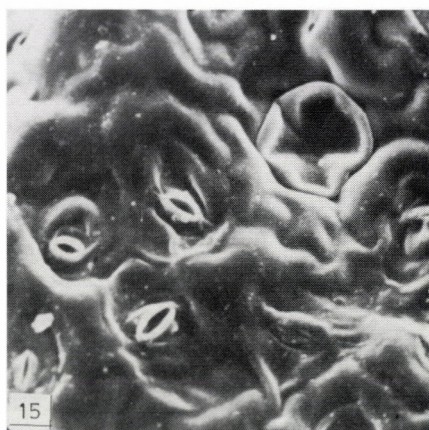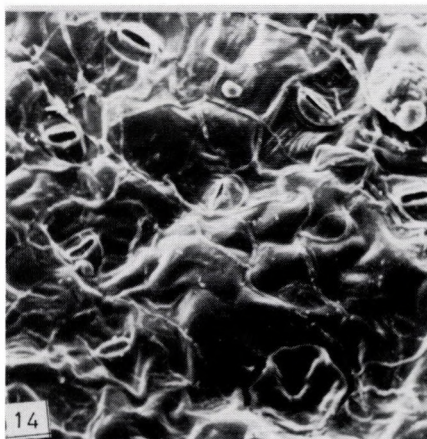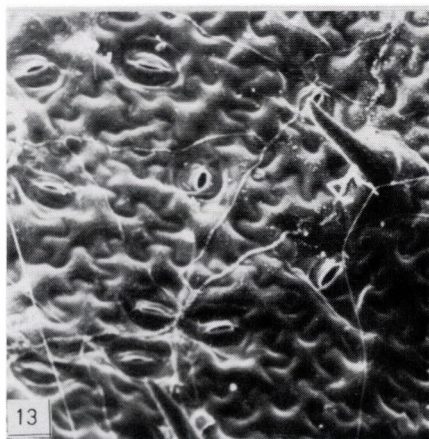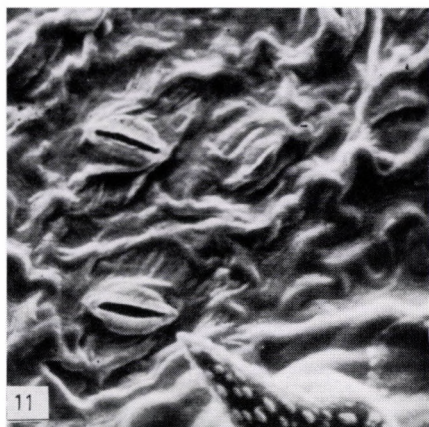
Plate III
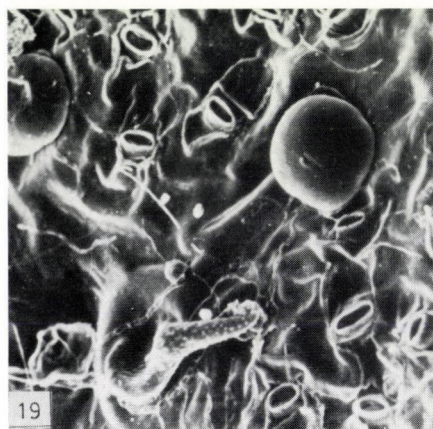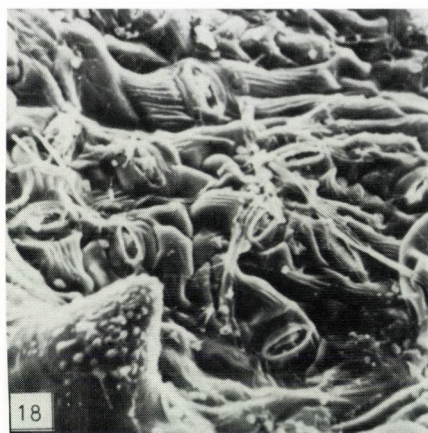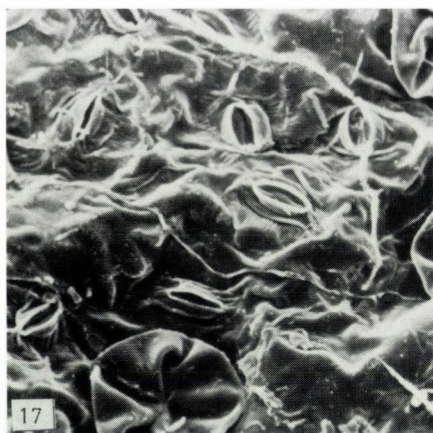
PLATE IV. Lower leaf surfaces

Figs 17—20. 17. C. lindenianum; Ch. WRIGHT 3177, x 540. — 18. C. lindenianum var. camagüeyense; Camaguey, ACUNA 13783, x 540. — 19. C. nipense; Moa, CLEMENTE 4961, x 400. — 20. C. nipense var. pubescens; Cristal, ALAIN et al. 5349, x 400

Plate V. Leaf surfaces of C. calcicola and C. tuberculatum

Fig. 21—26. 21. C. calcicola; PR. Cabo S. Antonio, BISSE et al. 30907, x 540 (low). — 22. C. calcicola; MAT, Zapata, HAC 33694, x 100 (low). — 23. C. tuberculatum; Sta. Clara, St. Spiritus, ALAIN 985, x 540 (low). — 24. C. tuberculatum; Escambray, L. FIGUEIRAS 243, x 100 (low). — 25. C. calcicola; PR. El Canimar, BISSE et al. 30904, x 540 (upp.). — 26. C. tuberculatum; Hab. Somorrostro, LEÓN 13654, x 540 (upp.)
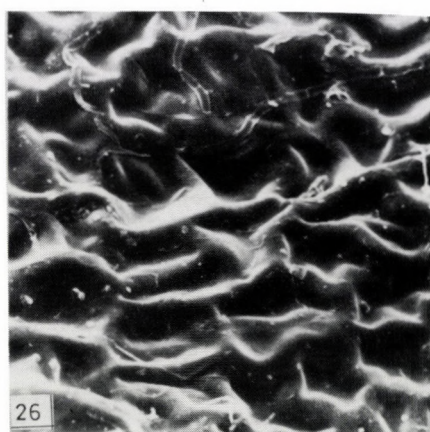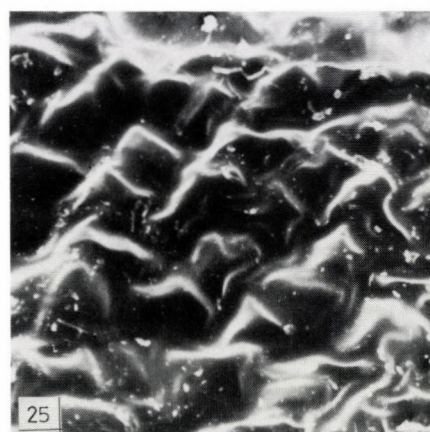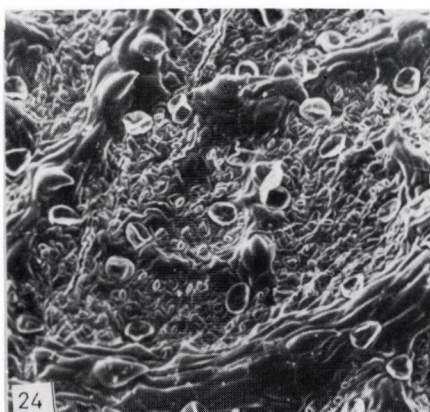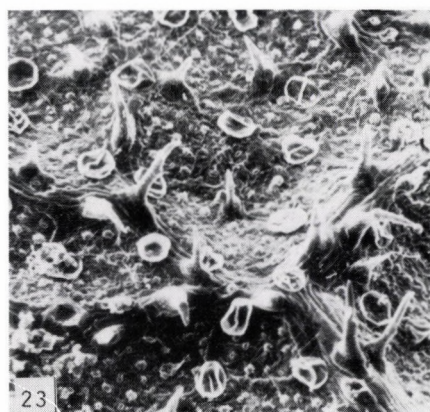
Plate V

The type 11 of many cells occur only in the Verbenaceae, where they were countered in 30 per cent of the genera but often in only one species per genus. Glands of similar construction were documented by ROBERT (1912) in some species of Clerodendrum and by CANTINO (1990: 355) in C. anafense. The leaves of the vast majority of Clerodenrum bear simple uni- or multicellular unbranched (= uniseriate) nonglandular hairs too. Branched multicellular hairs did not found in the taxa occurring in Cuba.

Results

C. aculeatum var. aculeatum

Upper leaf surface are provided with rare, scattered, rigid glandular hairs. Areola of the upper surface is shining, strigose with parallel micro-wrinkles. The cuticle of the lower surface more densely scabrous, flat. The areole and stomastructure of the forms occasionally different.

— f. acutifolium: Both surface densely glandular with a cross-shaped cut on the surface of the glands. Reticulation slender, some trichomes along the veins. Stoma typical, 20 μ long. Widespread in the whole Cuba and the Caribbean Islands (Fig. 7).

— f. mucronatum: Both surfaces are strongly reticulated, scarcely glandular. The lower surface is scabrescent, areoles provided with micro-reticulation. The stomata are typical, its length 19 μ. Described from a specimen collected in Santo Domingo.

— f. lanceolatum: Both surface weakly reticulated and scarcely glandular. The lower surface scabrescent, the upper glabrous. The cuticle bandles of the stomata (20 μ long) does not strigose. Frequent in Santo Domingo, rare in Cuba.

— — f. rotundatum: Reticulatio slender, scarcely harining and glandular on the lower surface. Stomata (18 μ long) conspicuously scarce with 2-3 radial cuticle-bundles (Fig. 8).

C. aculeatum var. gracile

Both surfaces densely scabrous and glandular.[1] The trichomes below are of various length and form even within the sample. Surface of the areoles characteristical "pebbled". Stomata typical 16 μ long. Their few bundles are straight. Frequent in the caribbean region (Fig. 9).

[1]Upper surface bear only scattered uni- or bicellular trichomes.
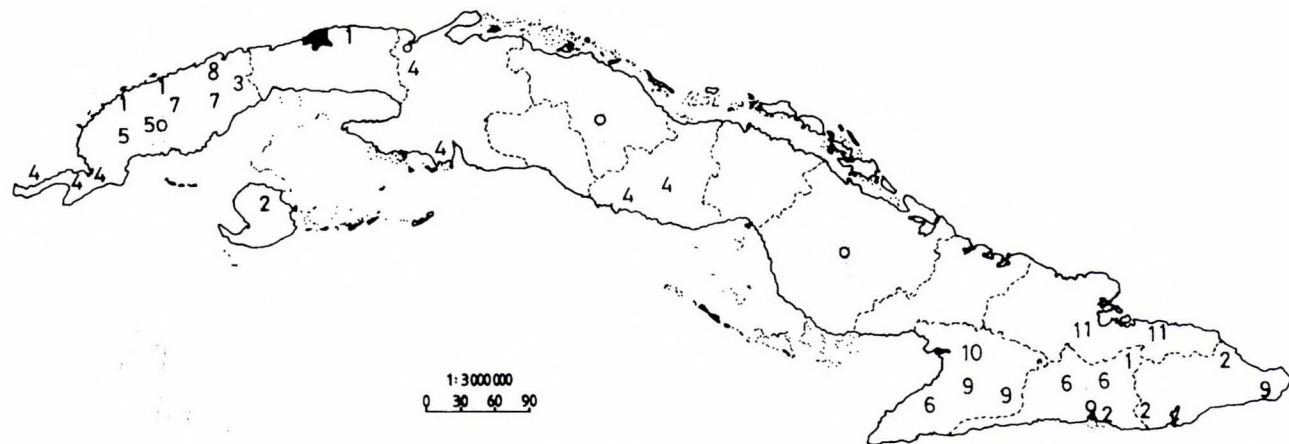
Fig. 27.

— — f. orientale: Upper leaf surface scabrescent with dense small thick[1] the lower surface villous (Fig. 10).

C. anafense

Cuticle on both surfaces slender undulating with strong reticulation. Areoles dull green, scarcely glandular or glabrous. On the main veins scattered bristles. Stomata of the anafense type, 18 μ long. Endemic in the Sierra de Anafe (Fig. 11).

C. cubense

Both surfaces glabrous or scabrescent with strong reticulation, on the lower surface with some short unicellular hairs. Cuticle protuberant, densely glandular. Stomata of cubense type, conspicuously small, 12 μ long. Endemic in the mountains of Cuba (Fig. 13).

— — var. brachypus: Unregular emergences of the cuticle are major, rougher, with densely small stomata (14 μ long) (Fig. 14).

C. denticulatum

The strongly double reticulated surfaces of the leaf densely glandular and scabrous with pitted, occasionally multicellular trichomes. Around the very small (12 μ long) stomata of the anafense type with conspicuous radial cuticle bundles. In some places of Central Cuba (Fig. 12).

C. grandiflorum

The lower surface green, shining, glabrous, slightly undulating with slender reticulation, densely glandular towards the leaf-apex. Around the stomata of nipense type (14 μ long) arise frequently a wrinkle-ring. Endemic in the mountains of W-Cuba (Fig. 15).

— — ssp. cajalbanense: It differs from the type by his more arised reticulation, scabrescent areoles and with more densely founded stomata. Along the veins are to be seen some potted unicellular glandular trichomes. Endemic on the serpentine forest of Cajalbana in NW-Cuba (Fig. 16).

C. lindenianum

Reticulatio slender, surface scabrescent. The inferior, still glabrous veins merge with the surface. The regular arised areoles densely glandular. The stoma-structure also irregular, transitory between the anafense- and nipense-types. Around the 15-19 μ long stomata mostly 2 cuticle-wrinkles. Endemic in prov. Oriente (Fig. 17).

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[1]Glandular trichomes and unicellular thin hairs as well.
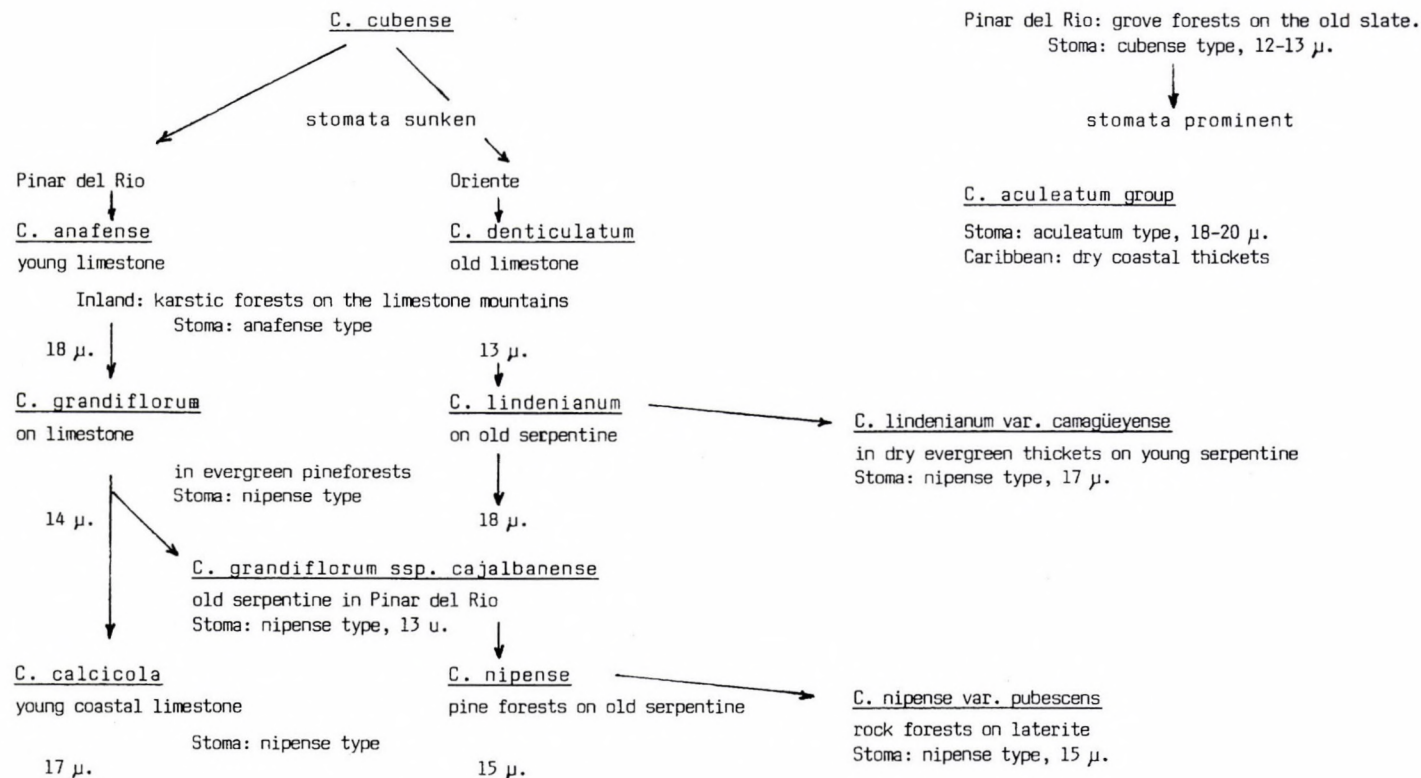
Fig. 28. Grouping of the species on the basis of development of their stoma-structure and locality

— — var. camagüeyense: A type differt with his irregular wrinkled areoles with short thick potted glandular trichomes and with his stomata near to nipense type, 17 μ long (Fig. 18).

C. nipense

The loose cuticle wrinkles provided with many small globular glands and scabrescent by short, thick, bulbed simple trichomes. The 16 μ long stomata are of nipense type. Endemic in the Sierra de Nipe (Fig. 19).

— — var. pubescens: The both surfaces villous compounded by mostly bicellular trichomes of various length. The 15 μ long stomata dispersed very densely. Living in both Sierra de Nipe and Sierra de Cristal (Fig. 20).

C. tuberculatum (C. calcicola)

The same epidermis structure on the micrographs also confirmed the previous suggestion having been this two taxa conspecific. The lower surface strongly reticulated, scabrous with many small, thick uni- or bicellular trichomes, the stomata of nipense type (17 μ long) are sitting in low pits densely spread on the surface. The micrographs present the lower and upper surfaces as well in Figs 21—27. The ecological character of the locality of C. calcicola (Peninsula Guanahacabibes) and C. tuberculatum (Central Cuba) are similar: littoral limestone.

Phylogenetical consideration

The diversity of the stoma- and epidermis-structure and their strong correlation with the site-ecological factors suggests a division oxplaining the potential evolution of the cuban representings of the genus on the basis of the stoma-evolution (Fig. 28).

The stoma- and epidermis-structure of C. cubense (cubense type) found here and there in the zonal semideciduous pine- and gallery-forests on the most ancient shale in Cuba, is the most simple and small of this art. Therefore is C. cubense to be considered as an ancient form widely spread on the total, still contiguous cuban territory. After the separating of two parts they remained on both two parts constituting the basis of the further evolution. The speciation could be continued in two direction. The first way may be the expansion of the genus towards the damp littoral-thicket. The stomata arised increasingly from the epidermis covered by the cuticle layer provided with densely hair under dry conditions. In this way, C. aculeatum group

living in littoral limestone-thicket have been separated. The second way is the expansion towards the inland seeking for their growing-space. Their stomata have been sunk gradually into the epidermis covered with thickened and wrinkled cuticle (anafense type). The stomata can be found among the loose, small wrinkles or often on the flat cuticle of the lower leaf surfaces of C. anafense living in the young karst forest of Sierra de Anafe (W-Cuba), and of C. denticulatum living in the semievergreen sclerophyllous forest on the limestone in prov. Oriente. In the next step of the evolution the cuticle layer have been strongly wrinkled, compounded tips for the embedded stomata (nipense type). This process run in the W-Cuban regions on the species living in limestone, proved by the separation of C. grandiflorum and C. calcicola. The geological difference effects only macromorphological changes on the individuals of C. grandiflorum ssp. cajalbanense. The same process went on in SO Cuba mainly on serpentine. C. lindenianum living here among the same conditions as that in Cajalbana have a transitory stoma-type to that of C. anafense. That is typical of both form, although the base form lives in pine forest, the variety in evergreen forest on young serpentine. Stomata of C. nipense group in laterite rock pine forest are densely spread and deeper embedded.

Acknowledgements

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APPENDIX

Specimens examined by SEM

C. aculeatum: PAULSEN 283 S Pinar del R., St. Princess; SCHWARTZ s.n. S, Jamaica; SCHWARTZ s.n. S, P. Rico, St. Bartholomew; ACUÑA 24579 HAC Oriente, Pico Turquino; EKMAN 15345 S, Santo Domingo: Pen. Samaná; ACUÑA 18280 Pinar del R. Lag. de Piedra; ALAIN 6052 HAC Pinar del R.

C. aculeatum var. gracile: MOLDENKE 19859 S Habana, Guanabacoa (Lectotype); BORHIDI 518915 BP Oriente, Sgo. de Cuba; BORHIDI 518805 BP Isla de P.; ACUÑA 17345 HAC Oriente, Baracoa; EKMAN 8253 S Oriente, Lag. de Bacona.

C. anafense: LEÓN 13654 HAC Habana; ALAIN 3227 HAC Oriente (Moa, C. cubense!); ROIG et al. 14057 HAC Habana, Sra. de Anafe; EKMAN 6499 S Pinar del R., Sra. de Anafe; WILSON 11466 HAC Pinar del R., Sra. de Anafe.

C. calcicola: ACUÑA 19933 HAJB Pinar del R., Guanahacabibes; BISSE et al. 30904 HAC Pinar del R., Guanahacabibes; BISSE et al. 30907 HAC Pinar del R., Guanahacabibes; ALAIN 985 HAC Las Villas, St. Spiritus (C. anafense var. tuberotrichum!); L. FIGUEIRAS 243 HAC Las Villas, Escambray.

C. tuberculatum: OVIEDO 33694 HAC Mat. Cienaga de Zapata; EKMAN 172 S Matanzas City; LEÓN 13654 Hab.: Somorrostro.

C. cubense: WRIGHT 3175 HAC (isotype: C. cubense var. brachypus); IMCHANITZKAJA 33604 HAC Pinar del R., Sra. del Rosario; EKMAN 16673 S Pinar del R., Sra. del Sitio Sto. Tomas; SAGRA 2866 KW.

C. denticulatum: FIGUEIRAS 2667 HAC Oriente, Palmarito de Cauto (topotype); EKMAN 9176 S Oriente, Pal. de Cauto (isotype); BORHIDI 517718 BP Oriente, Mogote Baire.

C. grandiflorum: WRIGHT 3176 HAC (isotype); ALAIN 6875 Pinar del R., Viñales; LEÓN 20974 HAC Pinar del R., Bahia Honda; BISSE et al. 291541 HAJB Pinar del R., Bahia Honda; BISSE et al. 30914 HAC Pinar del R., Pan de Guajaibón; EKMAN 17386 S Pinar del R., Playa Morrillo; SAGRA 2867 KW.

C. grandiflorum ssp. cajalbanense: ACUÑA 16416 HAC Pinar del R., Cajalbana; ACUÑA 16417 HAC Pinar del R., Cajalbana; ALAIN 24421 HAC Pinar del R., Cajalbana; YERO 575 HAC Pinar del R., Cajalbana.

C. lindenianum: WRIGHT 3177 HAC (isotype); ALAIN et al. 7390 HAC Oriente, Florida Blanca; ROIG 11761 HAC Oriente; LINDEN 1779 KW Oriente, Mt. Libanon; EKMAN 3991 S Oriente, Baracoa; EKMAN 6736 S Oriente, Nipe (C. nipense!); TURCZANINOW 2869 KW.

C. lindenianum var. camagüeyense: ACUÑA 13783 HAC Camagüey, La Cayería.

C. nipense: FIGUEIRAS 2591 HAC Oriente, Nipe; CLEMENTE 4961 HAC Oriente, Moa; WRIGHT 3175 HAC (isotype); EKMAN 3206 S Oriente, Nipe; EKMAN 9500 S Oriente, Nipe; LEÓN 22075 HAC Oriente, Nipe.

C. nipense var. pubescens: ALAIN et al. 5349 HAC Oriente, Sra del Cristal.

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THE EVOLUTIONARY MODIFICATION OF THE COROLLA AND ITS VASCULAR SUPPLY IN THE ASTERACEAE

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The major trend in the volution of the corolla is from actinomorphy to zygomorphy. Within the actinomorphic tubular corollas in the Asteraceae, the major trend has been the loss of dorsal bundles and finally even marginal bundles, so that the corolla has become totally non-vascular. In the final stages the corolla is either totally lacking or is presented by a short rim around the base of the style on the top of the inferior ovary.

Introduction

The Asteraceae, comprising about 900 genera and over 13 000 species are one of the largest family of flowering plants. The great homogeneity of the Asteraceae is displayed by their possession of the capitulum inflorescence, as well as the peculiar plan of their florets which even when divorced from the mother plant could in no case be mistaken from any thing else (GOOD 1956; LEPPIK 1960). To a student of evolutionary morphology of Angiosperms, the composite flower present several thought provoking problems. The nature of their pappus, placentation and inferior ovary are subject of acrimonious debate, which has already been reviewed in the earlier papers (TIAGI and SINGH 1972, 1981). The present work was undertaken with a view to investigate evolution of vascular supply of corolla in the family Asteraceae.

Material and Methods

In the present work, vascular anatomy of the flowers of 23 species of the family Asteraceae have been investigated. These are listed below:

0236-6495/93—94/$ 4.00

Sr. No.	Taxon	Source of collection
	TRIBE 1. VERNONIEAE	
1.	Vernonia cinerea (Linn.) Less.	Gwalior
	TRIBE 2. ASTEROIDEAE	
2.	Aster molliusculus Wall.	Mussoorie
3.	Brachycome assamica Clark	Chakrata
4.	Conyza japonica Less.	Mussoorie
5.	Cythocline purpurea (Don) Kuntze	Ujjain
6.	Erigeron multiradiatus Benth.	Mussoorie
	TRIBE 3. INULOIDEAE	
7.	Anaphalis cinnamomea Clark	Mussoorie
8.	Blumea lacera DC	Gwalior
9.	Blumea obliqua (L.) Druce	Gwalior
10.	Carpesium abrotanoides Linn.	Mussoorie
11.	Vicoa auriculata Cass.	Mussoorie
	TRIBE 4. HELIANTHOIDEAE	
12.	Coreopsis drumondii Tour. & Gray.	Ganganagar
13.	Glossocardia bosvallea (Linn. f.) DC	Dehradun
14.	Helianthus annuus Linn.	Ganganagar
15.	Lavia douglasii Hook. & Arn.	Jaipur
16.	Siegesbeckia orientalis Linn.	Dehradun
17.	Tithonia diversifolia (Hemsl.) A. Gray	Gwalior
	TRIBE 5. HELENIEAE	
18.	Tagetes erecta Linn.	Mussoorie
	TRIBE 6. ANTHEMIDEAE	
19.	Artemisia scoparia Waldst. & Kit.	Ganganagar
20.	Artemisia vulgaris Linn.	Mussoorie
21.	Cotula anthemoides Linn.	Ganganagar
	TRIBE 7. MUTISIEAE	
22.	Gerbera jamesonii Hook.	Gwalior
	TRIBE 8. CICHORIEAE	
23.	Cichorium intybus Linn.	Ganganagar

Customary methods of microtechnique were used. Serial transverse and longitudinal sections of the florets and capitula were cut at the thickness ranging from 7-15 µm. Double staining with crystal violet and erythrosin gave satisfactory results. In all cases the floral buds were cleared by warming in 10 per cent solution of potassium hydroxide and subsequently in colourless lactic acid. The cleared buds were dissected under a stereoscope. This was found to be very useful in understanding 3-dimensional picture of the vascular skeleton.

Observations and Discussion

In the family Asteraceae the presence of three types of corollas namely, tubular, ligulate and bilabiate has long been realized. Tubular type of corolla also includes the campanulate type, such as seen in Centaurea cyanus (SINGH 1973). In fact a more appropriate classification of the corolla types in the Asteraceae would be as follows:

I. Actinomorphic corollas

II. Zygomorphic corollas

(a) Ligulate type

(b) Bilabiate type

There can be hardly any doubt that the actinomorphic type of corollas are the most primitive among the family. SMALL (1917, 1919) expressed the opinion that the three types of corollas can be distinguished from one another by the variability of their anatomical characters. This statement is, of course, not very sound since great variation is seen in the vascular supply in each of the three types of corollas, from a condition of well-developed vascular supply to complete lack of vascular supply. In my opinion, the ligulate and bilabiate types of corollas are not related to each other but both are directly derived from the tubular type. A single deep sinus on the posterior side in the tubular corolla would give rise to the ligulate type, whereas deep sinuses in the postero-lateral position on either side would give rise to a bilabiate corolla.

The evolution of the vascular supply inside the corolla is to some extent related with the vascular pattern inside the wall of the inferior ovary has already been discussed at length in earlier papers (TIAGI and SINGH 1972, 1981). Undoubtedly, those corollas which possess five compound marginal bundles (cpm) and alternating with them five petal dorsal bundles (pd) are the most primitive for Asteraceae. Stenopadus of the tribe Mutisieae (CARLQUIST 1961) is one of the few composite where the ten traces inside the wall of inferior ovary after furnishing the stylar traces directly continue upward into the corolla. No such example is available in the present investigation among the disk-florets. The most nearly approaching condition is that of Helianthus annuus where ten bundles are present in the corolla tube (Fig. B). The condition in Tithonia diversifolia can be derived from that of Helianthus annuus by branching of each dorsal trace into three (Fig. A). This is a case of amplification, as a result of which the corolla tube contains in all twenty bundles, five triplets of three

bundles each, each triplet consisting of a dorsal bundle (pd) flanked with secondary marginal bundles (sm), alternating in position with the five compound marginal bundles (cpm). Amplification of the vascular supply in the corolla in the disk-floret is not a common feature of this family. The main evolutionary story of the vascular supply inside the corolla is one of reduction, either by suppression or cohesion. The dorsal bundles have been affected the most. Gradual stages in the total loss of all the five dorsal bundles are represented by taxa like Siegesbeckia orientalis, Layia douglasii, Cythocline purpurea and Vernonia cinerea (Figs C—F), to mention only a few.

Further reduction of the vascular supply inside the corolla of disk-floret includes reduction in the number of compound marginal bundles, either as result of fusion of the anterior two bundles into one as in Vicoa auriculate (Fig. H) or due to suppression of the posterior bundle as in Glossocardia bosvallea (Fig. G).

In the ligulate corolla on the other hand, both the processes of reduction as well as amplification have played a part in the evolution of its vascular pattern. The vascular pattern in Coreopsis drumondii is like the vascular pattern of the disk-floret of Helianthus annuus. The posterior compound marginal bundle of the petal splits up into its original constituents, followed by the splitting of the corolla between the two petal marginal (pm) bundles (Figs B and I). The condition in Siegesbeckia orientalis, Layia douglasii, Tagetes erecta, Brachycome assamica and Cichorium intybus (Figs J—N) is derived from that of Coreopsis drumondii by loss of one, two, three, four or all the five dorsal bundles. The condition in Aster molliusculus is derived from Cichorium intybus by loss of two marginal bundles in

→

Fig. 1. Diagrams (T.S. corollas) showing writer's views on the evolutionary modification of the corolla and its vascular supply in the Asteraceae.

Tubular corollas: A. Tithonia diversifolia, B. Helianthus annuus, C. Siegesbeckia orientalis, D. Layia douglasii, E. Cyathocline purpurea, F. Vernonia cinerea, G. Glossocardia bosvallea, H. Vicoa auriculata

Liguate corollas: I. Coreopsis drumondii, J. Siegesbeckia orientalis, K. Layia douglasii, L. Tagetes erecta, M. Brachycome assamica, N. Cichorium intybus, O. Blumea obliqua, P. Erigeron multiradiatus, Q. Anaphalis cinnamomea, R. Artemisia vulgaris, S. Blumea lacera, T. Artemisia scoparia, U. Carpesium abrotanoides, V. Conyza japonica, W. Cotula anthemoides, X. Aster molliusculus, Y. Gerbera jamesonii, Z. Gerbera jamesonii, AA. Helianthus annuus, BB. Tithonia diversifolia

Explanation for lettering: cpm, compound marginal bundle; pd, petal dorsal bundle; pm, petal marginal bundle; sm, secondry marginal bundle

Evolution of vascular supply of corolla
A
B
C
D
E
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BB
cpm
sm
pd
pm
pm
cpm
pd
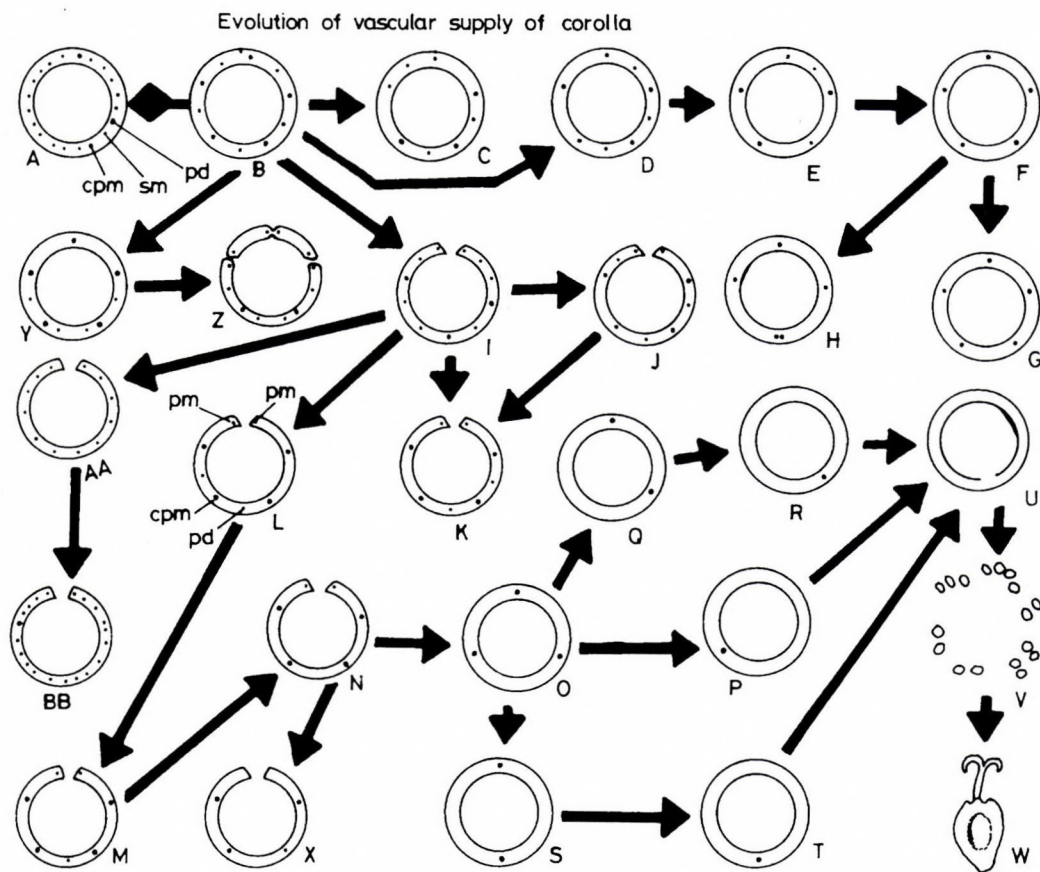
Fig. 1

the margins of the ligulate corolla (Figs N and X). The condition in Blumea obliqua, Erigeron multiradiatus, Anaphalis cinnamomea, Artemisia vulgaris, Blumea lacera, Artemisia scoparia and Carpesium abrotanoides is derived from that of Cichorium intybus by loss of one, two, three, four and all the five compound marginal bundles, so that the corolla becomes completely non-vascular (Figs O—U). The condition in Blumea obliqua is derived from Cichorium intybus by fusion on each side between an anterior and lateral compound marginal traces, so that only three bundles are seen in corolla. Similarly, the condition in Blumea lecera is derived from Blumea obliqua by further fusion of both the anterior compound marginal traces, so that only two bundles are seen in corolla. The next stage in the evolution of corolla is represented by Conyza japonica where the corolla is tubular and non-vascular in the lower part but higher up splits into a number of setose structures resembling a setose pappus (Fig. V). From the structure seen in Conyza japonica, reduction of the corolla to a short rim around the base of the style or complete suppression would give rise to the condition seen in Cotula anthemoides (Fig. W), which consists of the inferior ovary, style and stigma. Thus, in the line of evolution, we find reduction both of the vascular supply as well as of the corolla, form a condition where a corolla and its vascular bundles are fully developed to a condition where both are totally lacking.

In the other line of evolution illustrated by the ray-florets of Gerbera jamesonii, Helianthus annuus and both ray and disk-florets of Tithonia diversifolia (Figs Y—Z, AA—BB), there is an amplification of the vascular supply either by branching both of the dorsal and marginal bundles or by latter only.

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EFFECT OF ESTERS AND KETONES ON THE REDDENING OF DYER'S SAFFRON FLOWERS

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Seven esters and six ketones were fed to the flower pastes of dyer's saffron and their effects on the reddening were investigated by examining carthamin yield. Esters were more effective than ketones, indicating the following averaged promotion rate: 6.5 and 2.3% (2.8 : 1.0), respectively. In esters tested at 0.01—1000 mM level, phenyl methyl acetate was most promotive. Ethyl cinnamate came next. Ethyl linoleate followed this. Ethyl cyanoacetate showed a slight inhibition. Among ketones at the same concentrations, dimethyl ketone, ethyl methyl ketone and dibenzyl ketone exhibited promising capacities. Methyl vinyl ketone was found to be a strong inhibitor for the reddening of dyer's saffron flowers.

Introduction

The flower florets of Mogami-Benibana, a cultivar of dyer's saffron (Carthamus tinctorius), transcolours from orange-yellow to rusty-red at the phase of the influorescence. This transcolouration can be promoted in the presence of sugars (SAITO 1992a) and amino acids (SAITO and MATSUKURA 1993). As the colour induction proceeds readily under aerobic conditions (SAITO et al. 1983; SAITO and TAKAHASHI 1985), an oxidative process is considered to take part in the sugar or in amino acid-accelerated manifestation of the flower reddening. Recently, an experimental proof has been brought forward, indicating that glucose oxidase (EC 3.2.1.21) plays a leading role on the batho-shift reaction, where glucose is fed externally as the enzyme substrate (SAITO 1992a, 1993a, b).

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Abbreviations: BAW: n-butanol/acetic acid/water (4:1:2, v/v); HOAc: acetic acid/water (15:85, v/v); PW: phenol saturated with water; TLC: thin-layer chromatography.

The reddening reaction is not so simple as is usually supposed. It is directed by non-enzymatic (SAITO and TAKAHASHI 1985) and enzymatic processes (SAITO et al. 1983). The latter can be devided further into two sub-processes, namely direct (SAITO et al. 1985) and indirect (SAITO 1993a, b). These findings indicate that additional mechanisms might be involved in the flower colour transit reaction. On this view point, we expanded here our study program to other substances for detecting more efficient bathochronic colour inducers. In this study, effect of typical esters and ketones will be tested at various concentrations.

Material and Methods

Materials

Ethyl acetate, ethyl malonate, ethyl cinnamate, ethyl linoleate, methyl phenyl acetate, butyl acetate, ethyl cyanoacetate, dimethyl ketone, diethyl ketone, methyl ethyl ketone, methyl vynil ketone, adipin ketone and dibenzyl ketone were purchased from Wako Pure Chemical (Osaka, Japan), Silica gel and cellulose TLC plates were purchased from Merck (Darmstadt, Germany). Avicel cellulose was a product of Asahi Kasei Kogyo (Tokyo, Japan). Other chemicals and reagents used were all analytical grade of purity supplied commercially.

Plant material

The seeds of Mogami-Benibana were sown in the soil of our experimental field on April 28, 1993. After about three-month's cultivation, orange-yellow tubular flowers were harvested from the freshly opened flowering heads and frozen immediately at -20 ^{O}C in a freezer just before the experimental use.

Feeding of esters and ketones to flower paste

Each 0.5 g fresh flowers was crushed in 10 ml esters or ketones (0.01—1000 mM each) with a pocelain pestle and mortar for 5 min and resulting pastes left for 25 min at room temperature. The reddened pastes were used to the following experimental process.

Partial purification of red product

The red floral pastes from above process were suspended in 20 ml 0.5% (w/v) K_2CO_3 and stirred for 3 min on a magnetic stirrer, then the mixtures on a Büchner funnel filtered by suction. The alkaline extraction was carried out further twice and, at each repetition, fresh 20 ml 0.5% K_2CO_3 was added anew. The combined filtrates were acidified by the addition of 0.3 g citric acid and transferred to 50 ml glass tube, in which 0.5 g Avicel cellulose had been added. The tube contents were stirred with a glass bar and centrifuged for 5 min at 3500 rpm. The supernatant was discarded and the red pellet washed three times through centrifugation with each 50 ml distilled water.

Extraction of red product

The cleaned Avicel was suspended in 23-30 ml 60% (v/v) acetone, after a few minutes' stirring with a glass bar, the suspension was centrifuged for 5 min at 3500 rpm. The red acetone layer was retained and the acetone extraction repeated further 3-4 times, at each time, old acetone was replaced by new one. A net volume of acetone extracts thus prepared was applied to the following process.

Tentative identification and estimation of red product

A conventional chromatographic identification was carried out by using silica gel or cellulose TLC plates. The developing solvents used were: A. BAW, B. HOAc, C: PW. The migration distances were measured on the chromato-plates after drying in the air for several hours. The R_f-values thus measured were compared minutely with those of an authentic specimen which had been applied to both sides of the chromato-plates.

Product contents were quantified by using a Hitachi, model U-1100 spectrophotometer. The data were consulted with a calibration curve to determine the net content of the red product.

Results and Discussion

A flower reddening manifested under normal conditions in dyer's saffron capitula is reproducible experimentally (SAITO 1989). The reaction has been shown to be enhanced by the addition of sugars and amino acids (SAITO 1992; SAITO and MATSUMURA 1993). To accumulate additional evidence, we tested here seven esters and six ketones at 0.01—1000 mM concentrations if they increase red colouring matter contents in the flower paste of Mogami-Benibana. Before conducting the flower reddening tests, tentative identification was carried out using a product from the reddened flowers prepared by aqueous acetone. Table 1 lists the results from TLC. R_f-values of the red product and authentic carthamin are roughly coincident with each other. Thus, the acetone-induced product was identified as carthamin.

Table 1

Chromatographic identification of acetone-induced product

Solvent	R_f-value			
	Acetone-induced product		Authentic carthamin	
	Cellulose	Silica gel	Cellulose	Silica gel
BAW	0.197	0.687	0.186	0.687
HOAc	0	0.761	0	0.761
PW	0.061	0.328	0.058	0.343
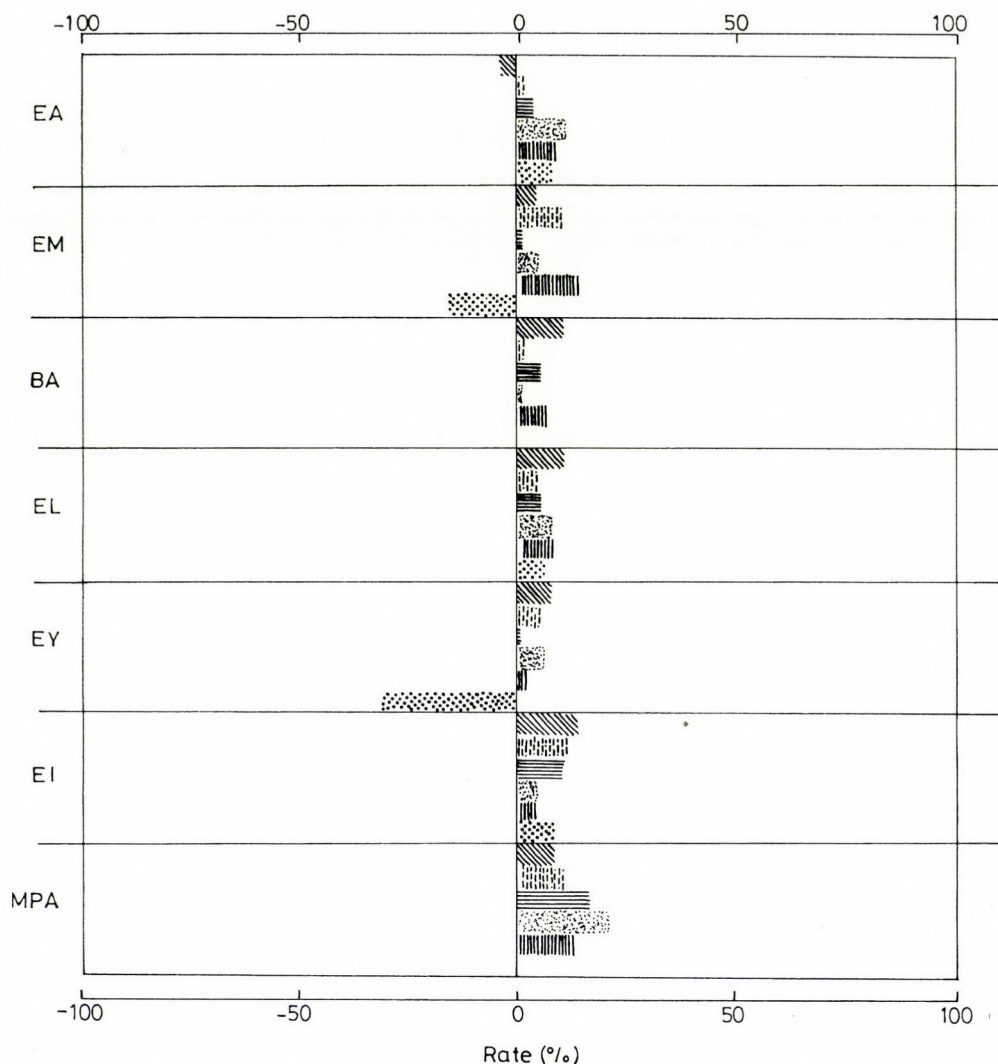
Fig. 1. Effect of esters on the reddening of dyer's saffron flowers. Seven esters were tested at various concentrations as indicated in the figure. ╲╲╲: 0.01 mM, ¦¦¦: 0.0 mM, ═══: 1 mM, ░░: 10 mM, ||||: 100 mM, ▒▒: 1000 mM. EA: ethyl acetate, EM: ethyl malonate, BA: butyl acetate, EL: ethyl linoleate, EY: ethyl cyanoacetate, EI: ethyl cinnamate, MPA: methyl phenyl acetate. -: inhibition, +: promotion

On mixing external esters and ketones in the triturated pastes of dyer's saffron flowers, red colouration develops slowly. However, the facilitation rate and its speed are different obviously by the compounds administered. Tables 2 and 3 summarize the data from the feeding experiments.
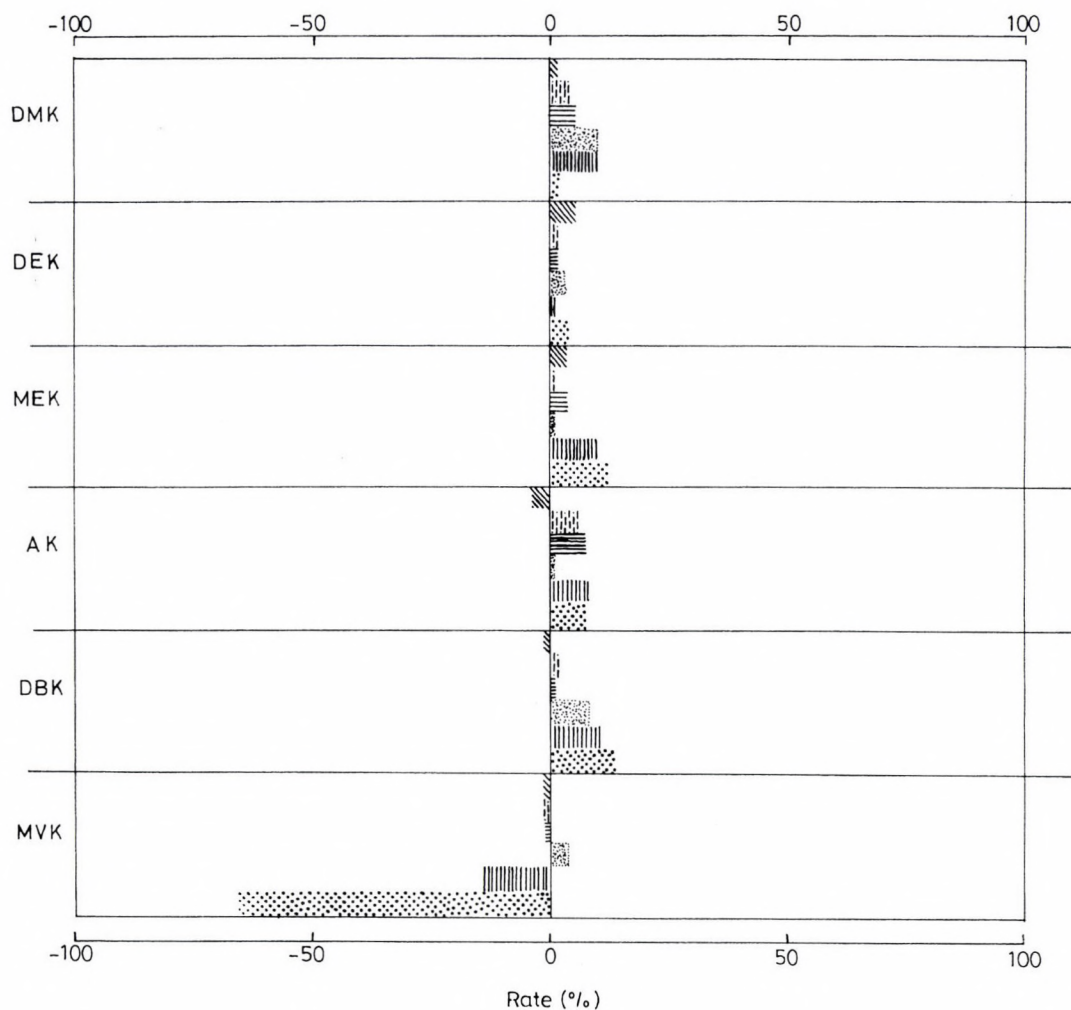
Fig. 2. Effect of ketones on the reddening of dyer's saffron flowers. Six ketones were tested at various concentrations as indicated in the figure. ▧: 0.01 mM, ▧: 0.01 mM, ▤: 1 mM, ▨: 10 mM, ▥: 100 mM, ▩: 1000 mM. DMK: dimethyl ketone, DEK: diethyl ketone, MEK: methyl ethyl ketone, AK: adipin ketone, DEK: dibenzyl ketone, HVK: methyl vynil ketone. -: inhibition, +: promotion

On the whole, esters promote the reddening process more effectively than ketones (promotion rate in average: 6.5 and 2.3%, respectively). Figures 1 and 2 illustrate finer details of the effects from the fed esters and ketones. Among the test esters, methyl phenyl acetate is most effective

Table 2

Effect of esters on the reddening of dyer's saffron flowers

Ester	Carthamin formed (ng carthamin/ml)*	Promotion rate (% of control)**
Ethyl acetate	215 ± 11.57	4.37
Ethyl malonate	216 ± 21.37	4.85
Butyl acetate	215 ± 9.26	4.37
Ethyl linoleate	222 ± 5.35	7.77
Ethyl cyanoacetate	202 ± 30.16	-1.94***
Ethyl cinnamate	223 ± 8.23	8.25
Blank	206 ± 8.74	

*, ** average values from testing at 0.01—1000 mM concentrations
*** inhibition

(11.2%): it affects most strongly at 10 mM dosage (18.2% increase). Ethyl cinnamate comes next, which exhibits a high activity at 0.1 mM (17.2%). Ethyl linoleate follows this (7.8%). It promotes the reddening at 100 mM most promisingly (17.1%). The effect of ethyl malonate is reduced further (4.9%), while, at 100 mM dosage, a projected effect is presented (18.4%). Ethyl acetate and butyl acetate also promote, but far lesser extent (both 4.4%). Ethyl cyanoacetate inhibits the bathochroma shift (average inhibition rate 1.9%), though the dosage at 0.01—100 mM level is rater promotive towards the reddening reaction by 4.3% in average. CN^- is known as a strong electron-chain inhibitor in the respiration process. Hence, the cyano-ions may play a determinative role in the inhibitory action, because the flower reddening is controlled by oxidative reactions (SAITO et al. 1983; SAITO 1992b). Regarding ketones, dimetyl ketone, dibenzyl ketone and methyl ethyl ketone facilitate the red colour shift with similar capacities (facilitation rate 5.8%). These ketones are most effective at different concentrations as follows (mM, %): dimethyl ketone (100, 17.4), dibenzyl ketone (1000, 18.0), methyl ethyl ketone (1000, 17.9). Adipin ketone reddens positively (4.4%). It reacts most actively at 100 mM level (17.2%). Diethyl ketone is far weaker contributor (2.4%), whereas it contributes strongly at 10 mM (16.8%). Methyl vinyl ketone, contrary to other ketones, acts inhibitorily at almost all concentrations tested (average inhibition rate 12.6%, see Table 3 and Fig. 2). At 100—1000 mM dosage, the reddening reaction is reduced by about 15 and 63%, respectively. The reason why it hinders the flower reddening so seriously is not known.

Table 3

Effect of ketones on the reddening of dyer's saffron flowers

Ketone	Carthamin formed (ng carthamin/ml)*	Promotion rate (% of control)**
Dimethyl ketone	218 ± 8.79	5.83
Diethyl ketone	211 ± 5.85	2.43
Methyl ethyl ketone	218 ± 9.91	5.83
Adipin ketone	215 ± 10.25	4.37
Dibenzyl ketone	218 ± 13.25	5.83
Methyl vinyl ketone	206 ± 50.87	-12.62***
Blank	206 ± 8.74	

*, ** average values from testing at 0.01—1000 mM concentrations
***inhibition

In conclusion, test ketones and even esters are not so effecitve as sugars (SAITO 1992a) and amino acids (SAITO and MATSUMURA 1993). Thus, the present data indicate the possibility that these compounds may concern, if no all, only partially with the flower colour modification manifested specifically in dyer's saffron at the phase of the influorescence. Here, we have presented that methyl vinyl ketone (3-buten-2-one) inhibitis the red colour shift strikingly (see Table 3 and Fig. 2). This new evidence is possible to do much for studying the mechanism of the flower colour transit reaction in the near future.

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THE EFFECTS OF DAYLIGHT INTENSITY AND OSMOTIC POTENTIAL OF MEDIUM ON THE VEGETATIVE GROWTH OF SPIRODELA POLYRRHIZA (LINN.) SCHLEID.

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Spirodela polyrrhiza (Linn.) Schleid was cultured under varied daylight intensities in mineral nutrient solution the osmotic potential of which was varied using polyethylene glycol (PEG) "6000". The growth was assessed in terms of different parameters.

By all parameters used growth was least at the osmotic potential of -600 kPa and greatest at -18 kPa in the half strength of the basal nutrient solution used. For fresh weight and number of lobes of the thalli, maximum growth was recorded at 50% daylight intensity at all levels of osmotic potential used, and minimum at 20%. However, dry weight and root length increased progressively from 20% to a maximum at 100% daylight intensity. All the above parameters of growth and chlorophyll concentration decreased progressively at decreasing osmotic potential of the medium down to -600 kPa; below this level the plants hardly survived. Chlorophyll content of thalli was miximum at 20% daylight intensity and minimum at 100%. The ratio of chlorophyll a:b decreased under low light intensities and under decrasing osmotic potential of the growth medium. The chlorophyll content at different osmotic potentials of the medium is a more consistent indicator of (dry weight) growth at low (20%) daylight intensity than at higher intensities.

Growth in terms of number of thalli was less consistent in relation to light intensity and osmotic potential of the medium.

Introduction

Spirodela polyrrhiza (Linn.) Schleid (Greater Duckweed) is the largest in size in the family Lemnaceae (duckweeds). Being a floating hydrophyte it is a suitable experimental material for physiological investigations. Its ability to multiply in hydroponic cultures and to float on top of water posing no aeration problems were recognized as special advantages by EVANS (1972). In addition LANDOLT and KANDELER (1987) restated the fact that the small size of duckweeds makes it possible to use a large number of individual plants in replicated samples. Also consequent on their small size they can be grown in relatively small space, thus minimizing logistic problems. All small-sized floating hydrophytes confer these advantages which

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have been exploited by many workers including WHITE (1936), NICKEL (1963) and JOY (1970).

Another attraction to the use of these floating hydrophytes is the ease of harvesting them and measuring their vegetative growth as fresh weight or dry weight. CLAPHAM (1965) also recognized the advantage of these plants being constantly in contact with water and air but not with soil. It does not readily strike the non-professional that duckweeds are flowering plants even though at a lower level of morphological differentiation than (say) a tree. To that extent their responses in given experimental situations could at least serve as a pointer to those of the other higher plants while advantage is taken of their size and habit to carry but a replicated study at much reduced expenditure.

Besides being used as a convenient experimental material some people (NYONG 1992) recognise a great promise and potential in duckweeds for several reasons. Their low ash and fibre contents as well as high protein content make them ideal for use in animal feed formulation. They are known to be relished by different kinds of birds, cattle and pigs; and even by people using them as vegetables in South East Asia including Burma, Laos, India and Thailand. They are also applied in the field as manure where they rival inorganic fertilizer. Moreover since they survive excellently in faecally polluted water and other environments rich in phosphorus, nitrogen and potassium, they might be used for cleaning polluted water. For these reasons, hydrobiologists, plant physiologists as well as weed scientists are interested in the conditions affecting or controlling the growth and proliferation of duckweeds.

EZE and DOLOR (1989) looked at the effects of the concentration of nutrient medium on the growth of Spirodela in a shaded environment. The osmotic effects of the medium concentration in their study could not be distinguished from those of the (salt) ionic concentration per se. In the present paper we present the results of a study which is an extension of that work. Osmotic stress in this case is applied by non-ionic means and in addition the prevailing light intensity is known percentage of the natural daylight in the open. How some selected parameters of growth in the hydrophyte change when both factors are varied simultaneously is the subject of this study.

Materials and Methods

The plant, Spirodela polyrrhiza (Linn.) Schleid (greater duckweed) used in this study was taken from the stock maintained in aquarium tanks for many years now in the Department of Botany, University of Benin. They were grown in water culture using the medium described by BOWKER et al. (1980), viz. $Ca(NO_3)_2$ $4H_2O$, 2×10^{-4} M; K_2HPO_4, 10^{-3} M; $MgSO_4 \cdot 7H_2O$, 2×10^{-4} M and KCl, 2×10^{-5}M. To each litre of the solution was also added one mL of micronutrients from the stock containing in $mgmL^{-1}$ MoO_3 (as molybdic acid), 170; H_3BO_3, 550; $ZnSO_4$, 30+ $MnCl_2 \cdot 4H_2O$, 180; $FeCl_3$, 550; $CoCl_2 \cdot 6H_2O$, 15; $CuCl_2 \cdot 6H_2O$, 2.5; $NaSiO_3$, 100; and EDTA, 2205. From preliminary trials half strenght of this formulation produced the best growth out of four different media tried.

For each experiment four replicate 500 mL plastic dishes containing 10 weighed thalli of the plant in 150 mL of the culture solution. Growth was measured at the end of 15 days. Parameters assessed were fresh weight, dry weight, length of three longest root per replicate dish, number of thalli, total number of lobes per thallus, and chlorophyll content.

Fresh weight was taken after drying wet plants quickly between sheets of filter papers. Increase in fresh weight was obtained as the difference between the final and initial fresh weights. Dry weight was obtained from plants dried in the oven at 70 ^{o}C for 24 h.

Chlorophyll was extracted from a known fresh weight of thalli by grinding with 80% acetone and centrifuging to obtain clear supernatant. Absorbance was measured at 645 nm and 663 nm and the calculation for the chlorophyll concentration was based on the method of ARNON (1949) for chlorophylls a, b and total chlorophyll. These measurements were made in triplicates.

The cultures were grown under daylight intensity which was varied by using different layers of mesh wire screens as described by EZE (1987), EZE and DOLOR (1989). The daylight intensities used were 20%, 50% and 100% (unshaded, full) daylight.

Osmotic stress was imposed by dissolving polyethylene glycol "6000" (PEG 6000) in the basal medium. The levels of stress were varied according to MITCHEL and KAUFMANN (1973).

Results

The fresh weight growth is best at 50% daylight intensity and minimal at 20% intensity. Under each daylight intensity growth decreases as the external osmotic potential becomes more negative (decreases). These observations are more clearly depicted in Fig. 1, where it is also obvious that the chlorophyll concentration of the thalli is highest under 20% daylight intensity and lowest under 100% intensity. At given osmotic potential values, the inhibitory effect on chlorophyll content or fresh weight production is not exerted in the same proportion under different daylight intensities.

The information summarized in Fig. 2 shows that the highest number of lobes is produced at 50% daylight intensity while the lowest number is obtained at 20%. This is also true of dry weight accumulation. On the other hand the root length is marginally favoured by 100% daylight intensity compared to 50% intensity. The root length development is also minimum under 20% daylight intensity. There is no obvious consistent effect of daylight intensity on the number of thalli. Once more in all the parameters evaluat-
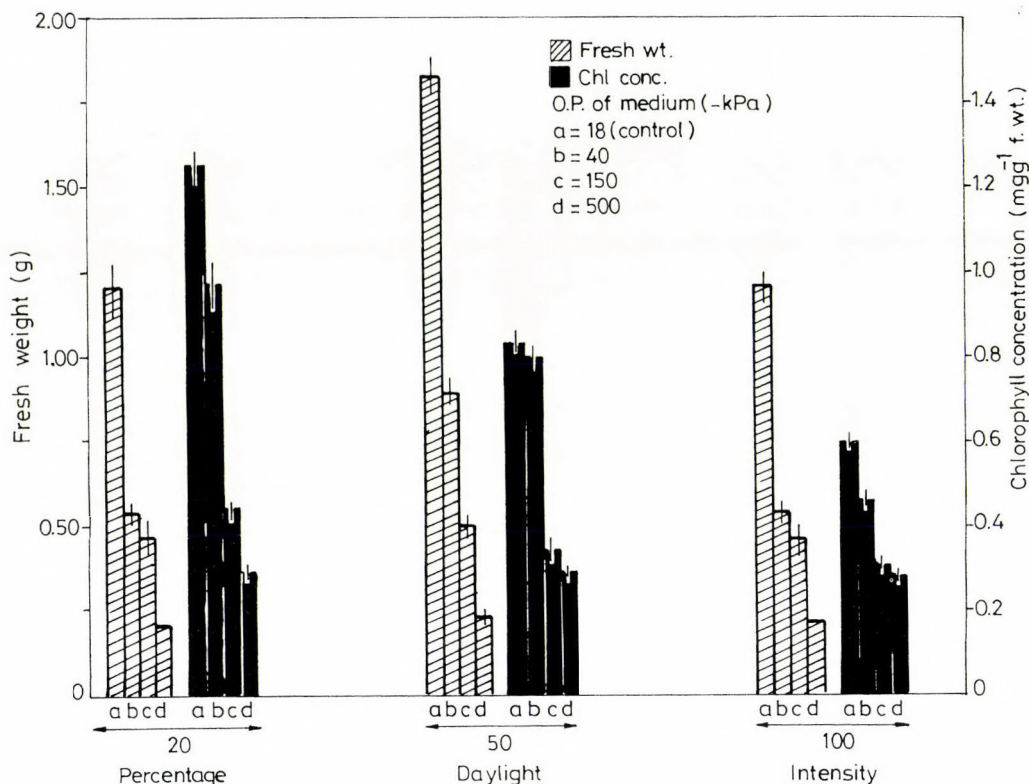
Fig. 1

ed, growth increases as the osmotic potential of the medium increases (becomes less negative) towards zero.

The ratio of chlorophyll a to chlorophyll b is more or less constant under 50% sunlight intensity at the different values of the osmotic potential of the medium. However, the ratio changes (generally decreasing as O.P. decreases) under 20% and 100% daylight intensities (Fig. 3).

Daylight intensity
□ = 20 %
△ = 50 %
○ = 100 %
No. of lobes
Dry weight (mg)
No. of thalli
Root length (mm)
-600 -150 -50 0
Osmotic Potential Medium (KPa)
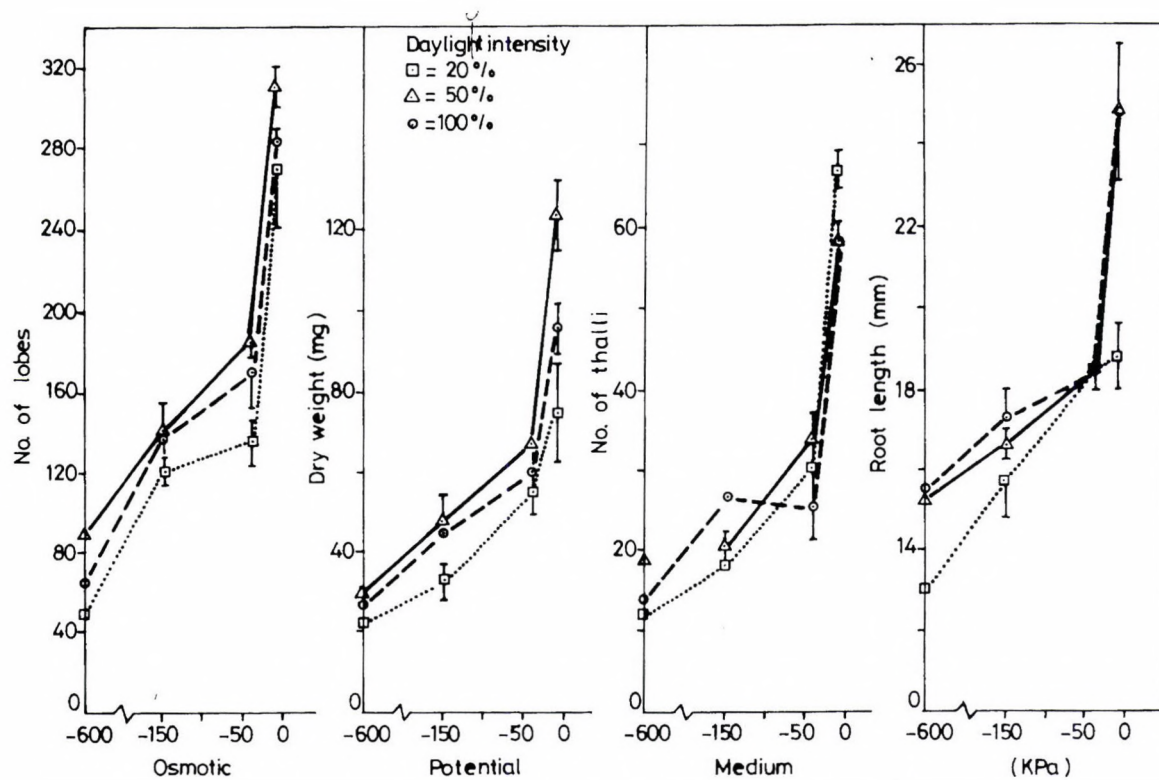
Fig. 2
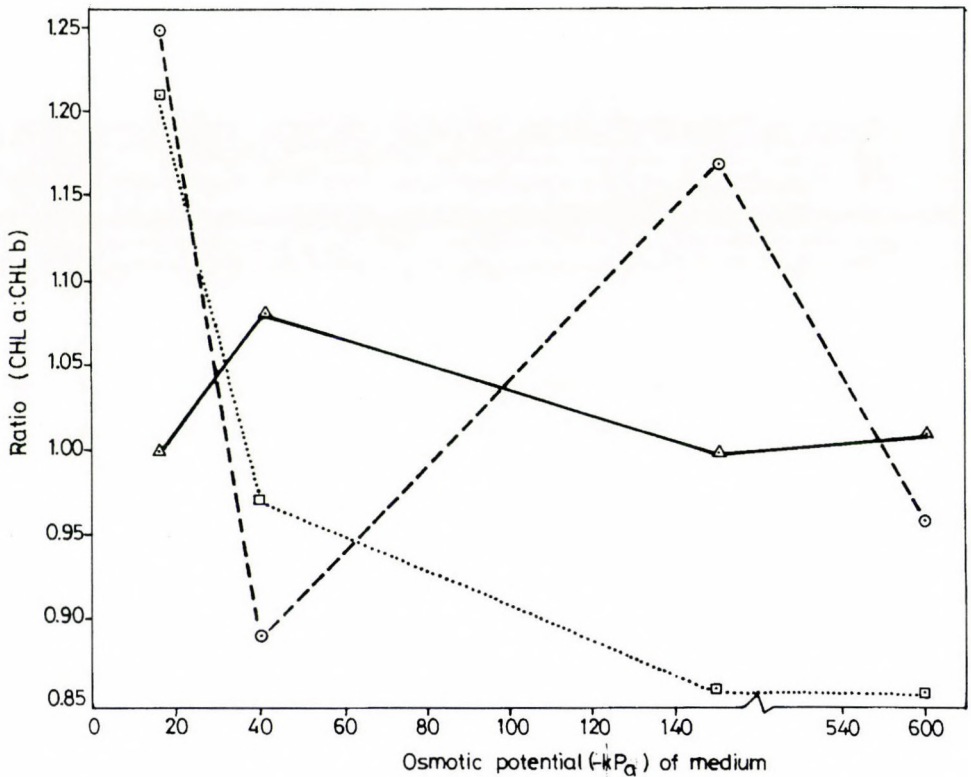
Fig. 3

Discussion

The results obtained in this investigation in which most growth parameters perform better below the maximum daylight intensity are in line with those of PATHAK (1970) on Tribulus terrestris; BRINKHUS and JONES (1974) on Chondrus crispus; and EZE (1987) on Amaranthus hybridus. AGAMI et al. (1980) also found that there was more growth in Najas marina at a few centimeteres below the water surface of the pond. In fact, ASHBY (1929) and SHIRLEY (1929) have observed that algae are not found growing at the upper surface of water owing to high daylight intensity. In sum the full daylight intensity is found to be supraoptimal for the growth of some plant species.

The above situation, however, cannot be generalized for all plant species. SAHID and JURAIMI (1990) have shown that Asystasia intrusa grows best at 100% sunlight compared to 47%, 31% or 19%. It is also clear from the re-

sults of Fig. 1 in the present investigation that root length growth was optimal at 100% daylight intensity. This means that the most reliable generalization to make in this respect is that the full (100%) daylight intensity is supra-optimal for most growth parameters in some plant species. Any claim that 100% intensity is optimal for growth in any species should be scrutinized or regarded with caution unless the investigation was exhaustive. For instance, the conclusion of SAHID and JURAIMI (1990) that 100% was best for the growth of Asystasia may be premature when the only other trials made were under 47%, 31% and 19% intensities. The intensities between 50% and 80% may be critical. The investigation of EZE and DOLOR (1989) with Spirodela growth under varying light intensities was inconclusive regarding full daylight intensity since the maximum light used was not in the open. The present investigation has resolved the uncertainty in this connection and makes it clear that Spirodela does not require full daylight intensity for optimal growth.

The observation that root length was maximum at 100% daylight has a precedent in the studies of MUENSCHER (1922), SHIRLEY (1929) and REID (1929): These workers have shown that a high level of light intensity favours increased development of roots relative to shoots in cereals, conifers and seedlings of herbaceous plants. WHITE (1936) also found that Lemna root length increased with increasing daylight intensity. He showed that root length was a sensitive indicator of the carbohydrate-nitrogen balance in the Thalli of Lemna.

The decreased chlorophyll concentration under 100% daylight was visually indicated by the pale-green coloration of the thalli. TRIBE and WHITTAKER (1971) and DANKS et al. (1983) have indicated that photosynthetic pigments undergo photo-oxidation at high light intensities. Unpublished data of EZE and MBENKUM on Lemna paucicostata shows even a much greater degree of paling of the fronds under full daylight intensity. The results in Figs 1 and 2 demonstrate that chlorophyll concentration is not a good parameter of growth as it neither varies directly or indirectly with fresh weight, dry weight, or other more obvious markers of growth performance. Since thalli do not bear a constant number of lobes, its number is also not a good parameter of growth. It is therefore more objective to count the total number of lobes whether the daughter thalli have separated from their parents or not. The number of lobes is a reasonable indicator of cell division and growth activity.

HALE and ORCUTT (1987) have stated that halophytes (salt-tolerant plants) have low osmotic potentials as a result of increased concentration of solutes. BUXTON, CYR and DUMBROFF (pers. comm.) interpreted their observed stimulation of root length growth in three North American coniferous seedlings as an effective drought-avoidance mechanism. They argue that a rapid increase in root length would permit the absorption of additional water from a physiologically dry environment. The above two instances refer and apply to halophytic and xerphytic situations, respectively. The case of Spirodela as a hydrophyte is different. The root length growth is highly sensitive to low osmotic potential. However, beyond mild osmotic stress (-150 kPa) the depressing effects of low osmotic potential reduces in intensity. In other words the effect is not proportional in a simple fasion. In fact, transforming the OP values to log scale for all the parameters evaluated converts the graphs to approximate straight line relationships.

An indication that both ontogenetic and environmental influences could be affecting chlorophylls a and b independently and thus be changing their ratios was first given by EZE et al. (1981). Working with Phaseolus vulgaris they demonstrated that although total chlorophyll was decreasing with advancing senescence, the ratio of a to b was rising steadily from age 5 to 28 days. In the present study there is a definite trend of decreasing chlorophyll a/b ratio at increasing osmotic stress under 20% daylight intensity. This trend is less obvious at 100% intensity and non-existent at 50% intensity. It is difficult to interpret these results rationally except to recognize that chlorophyll ratio changes under various influences. Whether the value is increasing or decreasing probably depends on the nature of the influence and identity of the species.

The examination of the problem in this study has revealed that both osmotic stresses and daylight intensities affect various aspects of the vegetative growth of Spirodela. The evidence so far suggests that these independent effects are non-interacting. It is possible to express the applied osmotic potentials as percentages of the value in the control experiment in a similar relationship to those of percentage daylight intensities. From this angle the osmotic effect will be seen as being more drastic than daylight intensity effects between 100% (control) and about 50% of each factor.

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AN OVERVIEW ON THE EFFECTS OF EXCESS CU ON RICE PLANTS

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A dose-response curve relating root growth to solution Cu concentration show that the rice root length is maximum with 150 nM of Cu. This metal tissue concentrations when compared with Cu concentrations in the nutrient solution ranging between 30 nM and 94 μM show two separate phases. Furthermore, the threshold toxic tissue concentrations as an average value of 35.1 μg/g /dw/ of tissue Cu. The concentrations of Cu in rice tissues show a sharp rise when Cu concentrations in a nutrient solution change from 30 nM to 94 μM, whereas the kinetics of Cu uptake during the 30 days after germination show a biphasic mechanism. Fe, Mn, N, P, K, Na, Ca, Mg, B, Mo, Zn and Al show heterogeneous root and/or shoot concentrations with increasing Cu toxicity, however the net translocation rate remains the same for each metal. In root cells excess Cu accumulates inside of the vacuoles, while in the shoots, at least in part, it accumulates in the vacuoles, and sticks or at least induces the accumulation of others chemical entities in the tonoplast. Furthermore, in the roots the amount of Cu seems to be related with Met and/or His concentrations, in 30 and 8.5 kDa proteins. Excess Cu decreases the activity of ACC synthase therefore limiting ethylene biosynthesis in both roots and leaves. Furthermore, in the roots it seems that the decrease of the biomass yield probably is affected by the sharp loss of protons from cells, being the growth of the shoots probably limited by o-diphenol, diamine oxidases and acid RNAase activities.

Introduction

It is long recognized that different plant nutrients show complex interactions (OLSEN 1972; EPSTEIN 1973), being high external Cu concentrations toxic to plants (FERNANDES and HENRIQUES 1991). BERRY and WALLACE (1989) suggested that in the physiological range of a metal solution, plant growth and absorption rates are in dynamic equilibrium, such that the metal tissue concentration will remain somewhere between the critical tissue concentration for optimal growth and the threshold toxic tissue concentration.

Abbreviations: ACC = 1-aminocyclopropane-1-carboxylic acid; EFE = Ethylene forming enzyme; His = Histidine; Met = Methionine; SAM = S-adenosylmethionine.

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A nutrient dose-response curve can be considered to consist of three phases corresponding to deficient, physiological and toxic concentration ranges of the nutrient (BERRY and WALLACE 1989). Accordingly the deficient range is that at which increased nutrient supply promotes root growth; the physiological range occurs when increasing concentrations of a nutrient do not affect growth; and in the toxic range, a nutrient increase induces a reduction in growth that is inversely proportional to the log of its concentration in solution (BERRY 1977). The dose-response curve that relates growth to the solution nutrient concentration can define two diagnostic points: the critical level for deficiency and the threshold for toxicity. The critical tissue concentration of a nutrient is usually a constant that results from a limited nutrient supply, toxic concentrations can cause unlimited passive nutrient uptake, even though normal growth has stopped (BERRY and WALLACE 1989). The nutrient-accumulation curve which relates the nutrient accumulation in tissue with nutrient concentration in the external solution can define a diagnostic criterion that determines the potential of a nutrient phytotoxicity. Nevertheless, it has been suggested that plant nutrient uptake, of either inorganic or organic ions, is mediated by a multiphasic mechanism (EPSTEIN 1973; BOWEN and NISSEN 1977; NISSEN 1974; SOLDAL and NISSEN 1978). Such proposal, originally introduced by NISSEN (1971, 1973), has been supported by subsequent studies carried out with a variety of metals, including Cu (BOWEN and NISSEN 1977; HASSAN and TANG VAN HAI 1976; OHKI 1975; VELTRUP 1977). VELTRUP (1977) showed the existence of a biphasic kinetic pattern for Cu uptake by roots of several species of Hordeum and that uptake can be strictly active or both active and passive (FERNANDES and HENRIQUES 1991).

Several plants growing in soils and waters have defense mechanisms against Cu toxicity and some of these species even show optimal growth at Cu levels that are lethal to other species (FERNANDES and HENRIQUES 1991). The excretion of Cu-complexing compounds that reduce metal availability in the soil or in the water, Cu exclusion through selective uptake of elements and this metal retention in the roots, preventing its translocation to the shoots, seems to be a widespread defense mechanism (FERNANDES and HENRIQUES 1991), occurring in some species as the intracellular compartmentation and precipitation of Cu in non-reactive forms. The increased production of intracellular metal-binding compounds (metallothioneins and/or phytochelatins or homo-phytochelatins) has also been reported (FERNANDES and HENRIQUES 1991). Nevertheless, the biomass yield in roots might be affected

throughout the interaction among uptake mechanism(s), plasma membrane-H^+ ATPase and root membrane permeability (HAGER et al. 1971; RAYLE and CLELAND 1977; CLELAND 1980; FERNANDES and HENRIQUES 1991). Furthermore, in the shoots the biomass production might be affected by the disfunction of the activities of ascorbate oxidase, diamine oxidase and o-diphenol oxidase (DAS et al. 1978; MACHLÁN and MINAR 1981; MALINSKI et al. 1985; FREDERICO and ANGELINI 1986; TORRIGIANI et al. 1989) as well as by un uncontrolled increase of the activities of acid RNAse and protease activities (KOWALSKI et al. 1974; DAVE and KANNAN 1980; NEURATH 1984).

This work is a revision of previous works on the effects of excess Cu on the rice biomass yield. Therefore, a global overview on the threshold of Cu toxicity, on the kinetics of this metal uptake and on the interactions with Mn, Fe, Zn, N, P, Na, Ca, Mg, B, Mo and Al absorption is produced. A subcellular localization of Cu in both root and shoot cells is also evaluated. Additionally, the ethylene biosynthesis in roots and leaves, the proton extrusion and membrane permeability in roots and the activities of ascorbate, diamine and diphenol oxidases as well as acid RNAse and protease in the shoots are reviewed and related with the biomass production of rice plants grown under increasing Cu concentrations.

Threshold of Cu toxicity

In a growth solution medium Cu concentrations higher then 30 nM promote in rice a progressive decrease of shoot elongation, during the 30 days following germination (LIDON et al. 1989; LIDON and HENRIQUES 1991a). Furthermore, rice shoots become chlorotic with 19 µM of Cu, whereas 94 µM induces a sharp necrosis. The elongation of rice roots also shows a progressive reduction, and with 19 µM in the growth solution medium the seminal root elongation become very small and is progressively replaced by a cluster of short, very hairy roots (LIDON et al. 1989; LIDON and HENRIQUES 1991a). Furthermore, 94 µM of Cu displays an absolute inhibition of secondary root formation, whereas the seminal root growth is also sharply inhibited (LIDON et al. 1989; LIDON and HENRIQUES 1991a). The biomass yield also sharply decrease in root and shoot tissues with Cu concentrations higher then 4 µM in the growth solution medium (LIDON and HENRIQUES 1992a, b). In rice a dose-response curve relating root growth to solution Cu concentration in a log-log plot show that the root length of rice is maximum with 150 nM of Cu

(LIDON and HENRIQUES 1992b, c). The Cu tissue concentrations when compared with the Cu concentrations in the solution show that tissue Cu concentration responds to solution Cu concentrations ranging between 30 nM and 94 µM by showing two separate phases (LIDON and HENRIQUES 1992b, c). In the first accumulation phase (associated with Cu solution concentrations up to 150 nM), tissue Cu concentrations increase was negligible. In the second phase (associated with Cu concentrations in the solution higher than 150 nM), tissue Cu concentrations showed a sharp rise. The transition point between the two accumulation phases occurred at a concentration of Cu in solution higher than would be expected when compared with published toxicity data (FERNANDES and HENRIQUES 1991; REUTER and ROBINSON 1987). When rice total tissue Cu concentration data is log-log plotted against root growth, the nutrient calibration curve shows that the threshold toxic tissue concentrations as an average value of 35.1 µg/g /dw/ of tissue Cu (LIDON and HENRIQUES 1992b, c).

Kinetics of Cu uptake

The concentrations of Cu increase in both rice roots and shoots with increasing levels of this metal in a solution growth medium (LIDON and HENRIQUES 1991b, 1992c, d, e). In rice shoots a slight increase might be observed until 19 µM, whereas a 5-fold increase can be found between 19 and 94 µM of Cu in the growth solution medium (LIDON and HENRIQUES 1992c, d). In roots, Cu concentrations increase linearly and with a high slope between 750 nM and 94 µM of Cu in the growth solution medium (LIDON and HENRIQUES 1993a). Cu uptake during the 30 days after germination show a biphasic mechanism (LIDON and HENRIQUES 1992a, f). The rice plants submitted to Cu concentrations in the growth medium ranging between 30 and 750 nM display an uptake kinetic (phase 1) which contrast to those treated with Cu concentrations ranging between 3.8 and 93.8 µM (phase 2). Furthermore, it seems that a transition on the mechanism of Cu uptake occurs in the range of Cu concentration between 750 nM and 94 µM (LIDON and HENRIQUES 1992a, f). In phase 1, as compared to phase 2, the values of $Vmax_{ap}$ are always considerably lower and remain practically constant (LIDON and HENRIQUES 1992a, f). In phase 2, the $Vmax_{ap}$ values increase during the first 20 days to remain pratically constant afterwards (LIDON and HENRIQUES 1992a, f). The values of Km_{ap} values are generally high in phase 1, in contrast to what occurs in phase 2. In this latter phase the Km_{ap} values show two distinct behaviours,

namely an increase up to 23 days followed by a levelling off (LIDON and HENRIQUES 1992a, f). The values of $Vmax_{ap}$ and Km_{ap} indicate the occurrence of a biphasic mechanism for Cu uptake, involving both active and passive transport systems. In phase 1 the high Km_{ap} values as well as the corresponding low $Vmax_{ap}$, indicate the existence of low affinity binding sites for Cu and the presence of an active mechanism. In phase 2 the Km_{ap} suggest a progressive decrease of Cu affinity for the binding sites or a possible inactivation of these. Considering that all metals show a great tendency to form stable associations with charged centers (SILLEN and MARTEL 1964; HEDRICH and SCHROEDER 1989), the Km_{ap} increase probably originates from an increasing disfunction of charged centers responsible for the active transport. From the apparent Vmax and Km values it seems that Cu is absorbed by an active mechanism up to concentrations of 750 nM in the nutrient solution, with a passive absorption mechanism progressively taking over for increasing concentrations of the metal.

Interactions between Cu and Mn, Fe, Zn, N, P, Na, Ca, Mg, B, Mo and Al

Increasing Cu levels in the nutrient growth medium affect the concentrations of Fe, Mn, N, P, K, Na, Ca, Mg, B, Mo, Zn and Al in rice roots and shoots (LIDON and HENRIQUES 1992c, f, 1993a). In roots, while Zn concentrations decrease with increasing Cu levels in the nutrient solution, Mo and K concentrations seems to increase until concentrations of Cu in the growth medium of 750 nM and 3.8 µM, respectively, decreasing afterwards (LIDON and HENRIQUES 1993a). Fe concentration decrease after 750 nM, whereas P concentration seems to show a sharp decrease only after 3.8 µM of Cu (LIDON and HENRIQUES 1993c, f). The concentration of N, Mg, Na, Ca, B, Mn and Al do not show a clear tendency with increasing Cu levels in the nutrient solution (LIDON and HENRIQUES 1992f). In shoots, while Zn concentrations decrease until 19.8 µM of Cu, K and N concentrations seem to increase until 750 nM and 3.8 µM, respectively, decreasing afterwards (LIDON and HENRIQUES 1992f). P, Mn and Fe concentrations in shoots seem to decrease after 750 nM of Cu, whereas Na increase after 3.8 µM of Cu (LIDON and HENRIQUES 1992c, 1993a). Ca concentrations do not seem to be affected by Cu levels in the medium, whereas Mg concentration decrease slightly until 19 µM of Cu in the nutrient solution (LIDON and HENRIQUES 1993a). The concentration of B and Mo do not show a clear tendency with increasing Cu levels in the nutrient solution

(LIDON and HENRIQUES 1993a). The concentration of Al show minimum values with increasing Cu concentrations which seems to be in agreement with previous works (FOY et al. 1978). Using the mean of Fe, Mn, N, P, K, Na, Ca, Mg, B, Mo, Zn and Al concentrations as well as the biomass yields of roots and shoots, the mean of these metals content per 100 roots and shoots can be determined under increasing Cu toxicity. The absolute content of all these metals (except fe and Al) per 100 roots or shoots exhibit its highest values with 3.8 μM of Cu (LIDON and HENRIQUES 1993a). Because the ratio between root and shoot biomass yields changes among the different Cu treatments (LIDON and HENRIQUES 1992a, b) only by adding the mean of these metals contents per 100 roots and shoots, can net uptake 30 days after germination be calculated. Net uptake per plant and for all these metals (excepting Fe and Al) occurs with 3.8 μM of Cu, which is a further evidence of a limiting point for Cu tolerance in rice (LIDON and HENRIQUES 1992c, 1993a). By calculating the ratio between the mean of the shoot metal contents and net uptake, the mean of these metals translocation rate can be determined, 30 days after germination, for the different Cu treatments. The regression output (computing the Y interception) using as variables independent and dependent the mean of these metals contents per shoot and the mean of net uptake for the different Cu treatments, indicates a tendency of these metals net translocation rates. Although these metals might show heterogeneous root and/or shoot concentrations with increasing Cu toxicity, the net translocation rate remains the same for each metal suggesting that Cu affects these metals concentrations in shoots mainly by changing the net uptake rate. Indeed the absolute amount of each of these metals that is translocated seem to be a function of its absolute content in the roots (LIDON and HENRIQUES 1992c, 1993a). Therefore, in long-term experiments the variations of these metal concentrations in the shoots result mainly from changes of these metals net uptake induced by increasing Cu levels.

Subcellular localization of Cu

In rice roots and shoots the retention of Cu seems occurs in a specific intracellular compartmentation. Indeed, in root cells Cu seems to accumulate inside of the vacuoles when the concentration of this metal in the solution medium is higher then 750 nM (LIDON and HENRIQUES 1992g). Furthermore Cu also seems to deposit inside of small vesicles in the cytoplasm,

which seemed to melt with the vacuoles (LIDON and HENRIQUES 1992h). Cu accumulation in rice roots is somewhat similar to that reported by DANIEL and CHAMBERLAIN (1981) for Amphora veneta. Indeed, it seems that Cu accumulates (at least partly) in the root vacuoles, possibly throughout its transport inside of small vesicles along the cytoplasm. As previously observed in the tolerant Becium homblei (REILLY 1972; FERNANDES and HENRIQUES 1991) in the roots, Cu concentration also seemed to be related with Met and/or His concentrations, in 30 and 8.5 kD proteins (as well as in 11 and 2 kD proteins when the Cu concentration in the growth medium is greater then 19 µM) (LIDON and HENRIQUES 1992h). The concentration of Cu in the 30 kD proteins show a 8.4 fold decrease when this metal concentration in nutrient solution changes from 150 nM to 94 µM, whereas in the 8.5 kD proteins a 2.5-fold increase might be observed between the 30 nM and the 19 µM. Furthermore, high Cu concentrations were also detected in the 11 kD and especially in the 2 kD proteins when this metal concentration in the nutrient solution reachs 94 µM. Furthermore, the amino-acid composition of the 30 kD Cu protein shows a 21.7- and 14.8-fold decrease in His and Met when the Cu concentration in the growth medium varies from 30 nM to 94 µM, whereas the 8.5 kD protein show a 34.9- and 6.7-fold increase (LIDON and HENRIQUES 1992h).

When rice plants are submitted to Cu concentration higher then 750 nM in the nutrient solution, in the shoot excess Cu occurs in a form or suborganellar distribution which does not inhibit acid phosphatase and cytochrome c reductase activities, at least in part it accumulates in the vacuoles, and possibly sticks or at least induces the accumulation of others chemical entities in the tonoplast (LIDON and HENRIQUES 1992g). Furthermore, on isolated vacuoles it was observed that on a protein basis an overall 1.65-fold increase might be detected for Cu concentration when the Cu concentration in the nutrient solution varies from 30 nM until 94 µM (LIDON and HENRIQUES 1992h). Nevertheless, it seems that excess Cu is sequestered, but only in a small extend, in the vacuoles, since although Cu might range 3000-fold extracellularly, and 29-fold intracellularly only a 1.6-fold increase occurs for Cu concentrations in the vacuoles. The activities of acid phosphatase and NADH cytochrome c reductase sharply increase when Cu concentrations varies from 30 nM until 94 µM (LIDON and HENRIQUES 1992g). These enzymes might be sharply inhibited by Cu(II), by complexing the substrate, by combining with active groups of the enzyme, or by reacting with enzyme-substrate complex (NEWMARK and WENGER 1960; ALVAREZ 1962; SHAW 1966; HASEGAWA et al. 1976; JUMA and TABATABAI 1977, 1988). Accordingly, as in rice shoots

the activities of acid phosphatase and NADH cytochrome c reductase are not inhibited, probably excess Cu accumulates in the vacuoles in non-reactive forms. Indeed, the concentration of SH groups as well as the ratio SH/Cu in the shoot vacuoles show a sharp increase with increasing Cu levels (LIDON and HENRIQUES 1992g). Therefore, according with SALHANY et al. (1978), DE FILIPPIS (1979) and NICHOLSON et al. (1980) probably Cu accumulated in the shoot vacuoles is inactivated throughout this metal affinity for sulf-hydryl groups.

Ethylene biosynthesis in rice roots and leaves

On a fresh weight basis, the activity of ACC synthase in rice root and leaf tissues decreased when the plants are submitted to Cu concentrations in the solution medium higher then 750 nM (LIDON et al., submitted). Furthermore, although a higher activity might be detected in the leaves, a sharper inhibition of this enzyme activity was also found in these tissues (whereas in the leaves a 6.1-fold decrease can be detected between 750 nM and 94 μM, in he root tissues a 1.6-fold decrease occurs).

The EFE is the membrane-bound enzyme (or enzyme complex) involved in the final step of ethylene biosynthesis (APELBAUM et al. 1981; YANG and HOFFMAN 1984). Furthermore, the participation of a transition metal in the opening of the cyclopropane ring has been suggested for the chemical oxidation of ACC (BOLLER et al. 1979; BALDWIN et al. 1985), for the degradation of ACC by free-radical-producing enzymes (VIOQUE et al. 1981; BOUSQUET and THIMANN 1984) and for the production of ethylene by plant extracts in vitro (KONZE and KWIATOWSKI 1981). Cu treated rice root and leaf tissues show an increase of its activity until the 19 μM in the nutrient growth solution (LIDON et al., submitted). As a 2.5- and 1.5-fold increase can be observed in root and leaf tissues, it seems that excess Cu stimulates this enzyme activity. Indeed, although it has been reported that EFE might be a soluble cytosolic enzyme (VERVERIDIS and JOHN 1991), the tonoplast and plasmolemma seems to be its subcellular location in vivo (GUY and KENDE 1984b; PORTER et al. 1986; BOUZAYEN et al. 1990; GALLARDO et al. 1993). Therefore, as in rice excess Cu deposits inside the root vacuoles (LIDON and HENRIQUES 1992h) as well as in the leaf tonoplast (LIDON and HENRIQUES 1992g), possibly this transition metal might act as a cofactor of this enzyme activity. Nervertheless, since the endogenous ACC decreases in both Cu treated root and leaf

tissues, in vivo this enzyme activity is limited, therefore limiting ethylene production. Indeed, ethylene evolution decrease after whent he rice plants are submitted to Cu concentrations higher then 750 nM in both root and leaf tissues (LIDON et al., submitted).

According with (ADAMS and YANG 1979; Yu et al. 1979; KONZE and KENDE 1979; YU and YANG 1980; KENDE and BOLLER 1981) it seems that in rice the ACC synthase is the ratecontrolling enzyme in the pathway of ethylene biosynthesis that is inhibited by excess Cu.

Proton extrusion and membrane permeability in roots

Rice plants submitted to Cu concentrations ranging from 30 nM to 19 μM in the nutrient growth medium show a 7.4-fold increase on the plasma membrane-H^+ ATPase activity (as measured throughout proton extrusion) (LIDON and HENRIQUES 1993a). Furthermore, rice roots show between 150 nM and 3.8 μM of Cu a slight decrease on membrane permeability, while a marked increase was observed afterwards until 94 μM (MOREIRA et al. 1992). The observed interactions among Cu uptake mechanism(s), plasma membrane-H^+ ATPase activity and root membrane permeability seemed to explain the variations of the root biomass yield triggered by excess Cu. It has been suggested (HAGER et al. 1971; RAYLE and CLELAND 1977) that a plasma membrane-H^+ ATPase, which pumps protons from the protoplast to the cell wall space, induces growth enhancement because this acidification lead to the loosening of the cell wall and it increases the cell volume (RAYLE and CLELAND 1977; CLELAND 1980). In rice Cu uptake is counter-balanced by proton extrusion, whereas a sharp increase might be observed on root membrane permeability when Cu concentrations higher then 3.8 μM are used in the growth medium (LIDON and HENRIQUES 1993a). Therefore while until the 3.8 μM of Cu the apparent slight decrease of membrane permeability might allow the acidification of the space between the cell wall and the protoplast (thus increasing root biomass production), afterwards the decrease of root biomass yield probably results from the sharp loss of protons from cells (triggered, at least in part, by the sharp increase of membrane leakage). Furthermore on long term, it seems to prevent their accumulation in the apoplast space (preventing the loosening of the cell wall) therefore inhibits root biomass production.

Activity of ascorbate, diamine and diphenol oxidases in rice shoots

In rice shoots no direct relationship between copper levels and phenol concentrations exists (LIDON et al. 1991). It seems that 30 nM of Cu in the nutrient solution stimulates phenol accumulation, whereas metals concentrations ranging between 150 nM and 3.8 μM promote a small increase on phenol content and higher Cu concentrations (19-94 μM) cause its decline. The activities of ascorbate, diamine and o-diphenol oxidases increase when Cu concentrations in the nutrient solution increase between 30 nM and 19 μM, decreasing afterwards (LIDON and HENRIQUES 1991c). The o-diphenol oxidase activity contrasts with ascorbate and diamine activities by showing only a slight increase until the 750 nM of Cu in the nutrient solution. It was noticed that although the increased ascorbate oxidase activity would provide additional intermediary molecules required for cellular synthesis (DELHAIZE et al. 1985; FERNANDES and HENRIQUES 1991) and thus for shoot growth, the increase of diamine oxidase activity or its activity product, the aldehyde, brought about growth inhibition (LOOMIS 1974; MALINSKI et al. 1981; TORRIGIANI et al. 1989). Indeed, the two enzymes had somewhat antagonistic effects on growth and, since both activities increase with copper levels, the negative effects of diamine oxidase on growth might overcame the positive one of ascorbate oxidase. Furthermore, an increase of copper toxicity was associated with an increase on membrane permeability of rice shoots, with a consequent destabilization of the tonoplast which may result in leakage of vacuolar compartmentalized reduced o-diphenol substrates (NABLE et al. 1988) into the chloroplasts containing the activated o-diphenol oxidase (TOLBET 1973; MAYER 1987; NABLE et al. 1988). Thus, as the resulting oxidation products of phenolics inhibited photosynthesis by binding to enzymes of the reductive photosynthetic carbon cycle (LOOMIS 1974), it is possible that this may also have contributed to the observed inhibition of growth (LIDON and HENRIQUES 1991c).

Protein contents in rice shoots

The protein content of rice chloroplasts and shoots decrease with Cu concentrations in the nutrient solution ranging between 150 nM and 94 μM (LIDON and HENRIQUES 1993b, d). As toxic Cu treatments do not induce N deficiency on rice the decrease in protein content on rice plants does not

result from limitations of N concentrations (LIDON and HENRIQUES 1993a). Furthermore, the activity of rice shoot protease did not respond linearly to an increasing Cu concentration (LIDON and HENRIQUES 1993b). However, when compared to the Cu treatment submitted to 150 nM, the protease activity of the Cu treatment subjected to 19 μM showed a 2-fold increase and that of 94 μM showed a 4-fold increase, which suggested that high toxic Cu levels might lead to a decrease in the content of protease inhibitors resulting in the large stimulation of the observed protease activity (NEURATH 1984). In the shoots acid RNAse activity increase slightly when Cu concentration in the nutrient solution increase from 150 nM until 3.8 μM; however, the application of 19 and 94 μM of Cu when compared with the use of 150 nM of Cu in the nutrient solution, showed a 6-fold and a 8-fold increase of that activity, respectively (LIDON and HENRIQUES 1993c). The data suggested that an increase in shoot Cu content is related to an increase of acid RNAse activity. As the enhancement of acid RNAse activity is related to an increase in membrane permeability (DAVE and KANNAN 1980), probably an increase of Cu content promotes an alteration, or even a disruption, of the rice shoot cellular membranes which, in turn, is responsible for the observed increase in acid RNAse activity (LIDON and HENRIQUES 1993b).

Conclusion

Rice tissue Cu concentration responds to solution Cu concentrations ranging between 30 nM and 94 μM by showing two separate phases. With Cu solution concentrations up to 150 nM tissue Cu concentrations increase is negligible, whereas higher Cu concentrations in the nutrient solution promote a sharp rise in tissue Cu concentrations. Nevertheless, when rice total tissue Cu concentration data is log-log plotted against root growth, the nutrient calibration curve shows that the threshold toxid tissue concentrations as an average value of 35.1 μg/g /dw/ of tissue Cu. Fe, Mn, N, P, K, Na, Ca, Mg, B, Mo, Zn and Al show heterogeneous root and/or shoot concentrations with increasing Cu toxicity, however the net translocation rate remains the same for each metal suggesting that Cu affects these metals concentrations in shoots mainly by changing the net uptake rate. Indeed the absolute amount of each of these metals that is translocated seem to be a function of its absolute content in the roots. In root cells Cu seems to accumulate inside of the vacuoles when the concentration of this metal in

the solution medium is higher then 750 nM. Furthermore Cu also seems to deposit inside of small vesicles in the cytoplasm, which seemed to melt with the vacuoles. In the roots, Cu concentration also seems to be related with Met and/or His concentrations, in 30 and 8.5 kDa proteins (as well as in 11 and 2 kDa proteins in the 6.25 mg/l Cu treatment), being possible that this metal excess forms complexes with these amino acids thereby being immobilized and probably transported to the vacuoles or that at least promotes the synthesis of enriched Met and/or His proteins not directly related with Cu co-ordination. In the shoots excess Cu occurs in a form or suborganellar distribution which does not inhibit acid phosphatase and cytochrome c reductase activities, at least in part it accumulates in the vacuoles, and sticks or at least induces the accumulation of others chemical entities in the tonoplast. Furthermore since this Cu accumulation does not inhibit acid phosphatase and NADH cytochrome c reductase, it is possible to assume that this metal excess is inactivated by its affinity for sulfhydryl groups (therefore inducing the oxidation and cross-linking of protein thiols).

Excess Cu in both root and leaf tissues decrease the conversion of SAM to ACC through the inhibition of the total ACC synthase activity. Furthermore, in the roots it seems that the decrease of the biomass yield probably is affected by the sharp loss of protons from cells (triggered, at least in part, by the sharp increase of membrane leakage), whereas the growth of the shoots might be negatively affected by o-diphenol and diamine oxidases. In rice shoots the protein concentration also seems to be affected by the increase of acid RNAse activity triggered by increasing membrane permeability.

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CATALASE, PEROXIDASE AND POLYPHENOLOXIDASE ACTIVITIES DURING ATTACHED RICE LEAF SENESCENCE UNDER NaCl-STRESS

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Changes in the activities of catalase, peroxidase and polyphenoloxidase were studied during the senescence of attached leaves of rice (Oryza sativa L.cv. Ratna) subjected to NaCl-stress. Chlorophyll and protein contents of the attached leaves decreased with time during the senescence process which were accelerated by NaCl-stress and retarded by kinetin. Catalase activity initially increased slightly up to 2 d and then declined up to the end of the experimental period. The declining trend of this enzyme activity was accelerated by salinization and decelerated by the application of kinetin in both non-stressed and NaCl-stressed leaves. Peroxidase, on the other hand, showed a trend in its activity of increasing spurtively up to 4 d and then decreasing up to 8 d which was retarded by NaCl-stress but accelerated by kinetin in both non-stressed and salt-stressed leaves. Interestingly, polyphenoloxidase exhibited a reverse trend in its activity to that of the peroxidase in both the non-stressed and NaCl-stressed leaves. However, kinetin could retard the trend of the activity of the former enzyme in both the type of leaves. The degree of leaf senescence under NaCl-stress is closely correlated with the changes in the catalase activity but not with peroxidase or polyphenoloxidase activities. However, the latter two enzymes exhibit a correlation inbetween their activities. The results suggest that salt-stress does not accelerate, and kinetin does not retard, all the biochemical processes during the senescence of attached leaves. Further, catalase activity can be taken as an indicator of attached leaf senescence under NaCl-stress, but not peroxidase or polyphenoloxidase activities.

Introduction

The adverse effect of salinity stress on the metabolism of plants is well documented (GREENWAY and MUNNS 1980; YEO 1983; YEO and FLOWERS 1986; GLENN et al. 1992). A good number of reports are available concerning the alterations in biochemical levels (MOFTAH and MICHEL 1987) and enyzmatic activities (YEO and FLOWERS 1986; ZISKA et al. 1988) in salt-stressed leaves. Enhancement of leaf senescence by NaCl salinity has been reported by a few (PRISCO and O'Leary 1972; KANG and TITUS 1989).

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We have reported alterations in three enzyme activities during senescence in detached rice leaves under NaCl-stress (SAHU and MISHRA 1987). In this investigation, during the process of senescence, catalase, an oxyradical scavenger tetrameric enzyme responsible for remival of relatively long-lived H_2O_2, a potent toxic in biological systems, exhibited a decreasing trend in its activity in the non-stressed excised leaves which was arrested under salt-stressed condition. Another oxyradical scavenger monomeric enzyme, peroxidase which utilises hydrogen peroxide, showed an increasing trend in its activity in the senescing detached leaves which was further increased under salt-stress. Polyphenoloxidase, which oxidizes the toxic phenolic compounds of the cell has been studied in relation to its increasing activity in senescing leaves (KAR and MISHRA 1976; PATRA and MISHRA 1979), but not yet investigated in salt-stressed leaves. Moreover, these oxidizing enzymes play important roles during the process of senescence, as aging in plants is accompanied by a progressive shift from a reduced to an oxidative state in tissues (CHOUDHURI 1988). In view of the importance of these enzymes, the present investigation has been undertaken to study the effect of NaCl-stress on the activities of these three enzymes in attached leaves of rice, a salt-sensitive cereal, during the process of senescence. An attempt has also been made to study the role of kinetin in regulating the activities of these enzymes in the salt-stressed leaves in attached condition.

Material and Methods

Seeds on rice (Oryza sativa L.cv. Ratna) were obtained from the Central Rice Research Institute, Cuttack. Uniformly healthy seeds were grown in earthenware pots containing sandy loam soil and green manure in the ratio 3 : 1. The pots were watered regularly till full saturation of the soil. On day 21, when the third leaves were seen to be matured, the pots were taken for senescence studies under salt-stress. For imparting salt-stress, some pots were irriaged with 50 mM NaCl solution and distilled water in alternate days till full saturation of the soil as this salt concentration has been previously reported to reduce by 50% the grain yield of many varieties of rice and also caused varying degree of mortality at the seedling stage (YEO and FLOWERS 1986 and references cited therein). The control pots received only distilled water daily. The leaves in some of the pots under salinization and without salinization were robbed smoothly with cotton pads saturated with 5 μm kinetin (6-furfuryl aminopurine) solution daily as this concentration of kinetin was shown to be most effective in a preliminary experiment. To induce senescence of the leaves, all the pots were kept in dark at 27 ± 2 °C from the beginning of the treatment up to 8 days. The third leaves of these plants from different test pots were cut 7 cm from the tip of the leaves, initially and in alternate days for biochemical and enzymatic analyses.

Extraction and estimation of the chlorophyll and protein and the assay of the activities of catalase (EC 1.11.1.6) and peroxidase (EC 1.11.1.7) were done as described previously

(KAR and MISHRA 1976), where as the extraction and assay of the polyphenoloxidase (EC 1.10.3.1) activity was done as described by PATRA and MISHRA (1979).

All the experiments were carried out thrice each with three replicates. The results showed almost the same trend in each case. The data presented in the figures are the mean value of the results obtained from the experiment. Standard deviations are shown in vertical bars. Regression analysis has also been presented.

Results

Chlorophyll content showed a decreasing trend in the non-stressed leaves which was accelarated in the NaCl-stressed leaves during attached leaf senescence incubated in dark (Fig. 1A). Treatment of kinetin, a powerful plant growth regulator, retarded this decreasing trend of chlorophyll levels in both non-stressed and salt-stressed leaves which was more pronounced in the latter. The level of protein in the non-stressed and salt-stressed leaves (Fig. 1B) changed in a similar manner as that of the chlorophyll, but the degree of changes in all the test samples was pronounced less in the case of protein than in the chlorophyll.

Catalase activity initially increased slightly up to 2 d and then decreased up to the end of the experimental period (Fig. 2). The decreasing trend of this enzyme activity was accelerated by salinization and at any given time, it (enzyme activity) remained at a lower level in the stressed leaves than that in the control. However, kinetin treatment retarded the declining trend of this oxyradical scavenger enzyme in both non-stressed and salt-stressed leaves and even up to 2 d the activity of this enzyme remained at a higher level.

Peroxidase exhibited a trend in its activity of increasing sharply up to 4 d and then decreasing up to 8 d during senescence (Fig. 3A). However, the increasing trend of this enzyme activity was retarded by NaCl-stress and accelerated by kinetin. At any given time, peroxidase activity remained at a lower level in the stressed leaves than in the control, and this was just the opposite in case of kinetin treatment where the enzyme activity was higher in both non-stressed and salt-stressed conditions. On the other hand, polyphenoloxidase showed a reverse trend in its activity (Fig. 3B) to that of the peroxidase in both non-stressed and NaCl-stressed senescing rice leaves. However, kinetin could retard the decreasing trend of the polyphenoloxidase activity in both the type of leaves. Further, this enzyme activity in the kinetin-treated leaves remained at a higher level than in the non-stressed or salt stressed leaves throughout the experimental period and the effectiveness of kinetin was more pronounced under stressed condition.
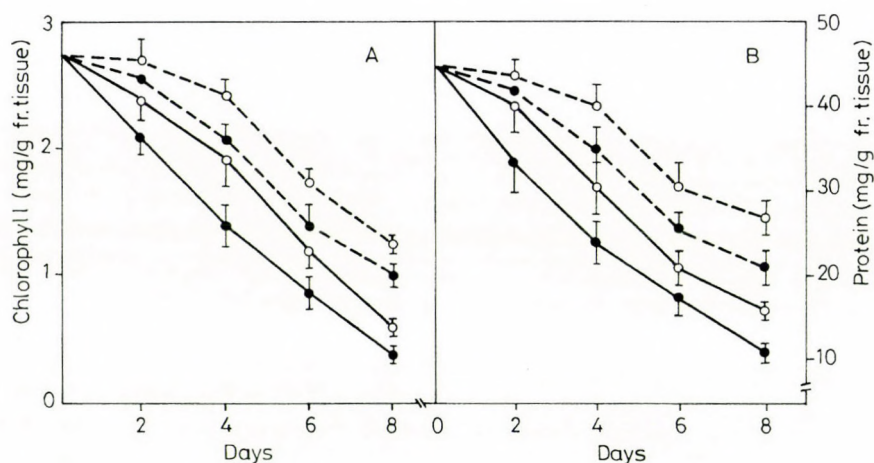
Fig. 1. Changes in the chlorophyll content (A) and protein content (B) of attached senescing rice leaves in non-stressed and NaCl-stressed conditions.
Symbols: o——o, non stressed (control) leaves; •——•, NaCl-stressed leaves; o- - -o, kinetin treated non-stressed leaves; • - - -•, kinetin treated NaCl-stressed leaves
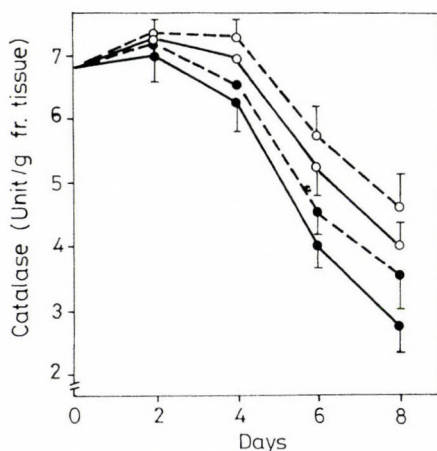
Fig. 2. Changes in the catalase activity (1 unit = mMH_2O_2 min^{-1}/g fr. tissue) of attached senescing rice leaves in non-stressed and NaCl-stressed conditions. Symbols as in Fig. 1

Regression analyses (Fig. 4) indicated that the degree of leaf senescence as measured by the loss of chlorophyll is significantly correlated with the catalase activity ($r = 0.9521$, $n = 16$ at $p = 0.001$), but neither correlated with peroxidase ($r = 0.3195$, $n = 16$) nor with polyphenoloxidase ($r = -0.3433$, $n = 16$) activities. However, a significant correlation
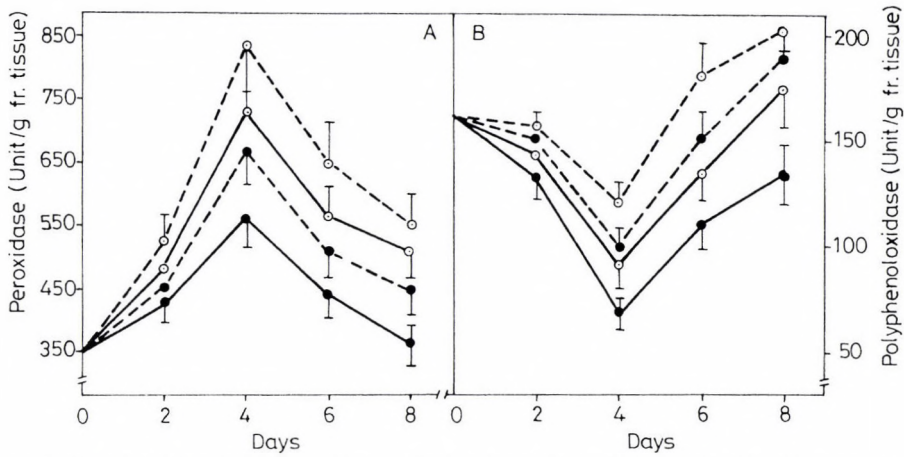
Fig. 3. Changes in the activities of peroxidase (A: 1 unit = ΔA_{420} by 0.1/g fr. tissue) and polyphenoloxidase (B: 1 unit = $.A_{420}$ by 0.1/g fr. tissue) of attached senescing rice leaves in non-stressed and NaCl-stressed conditions. Symbols as in Fig. 1
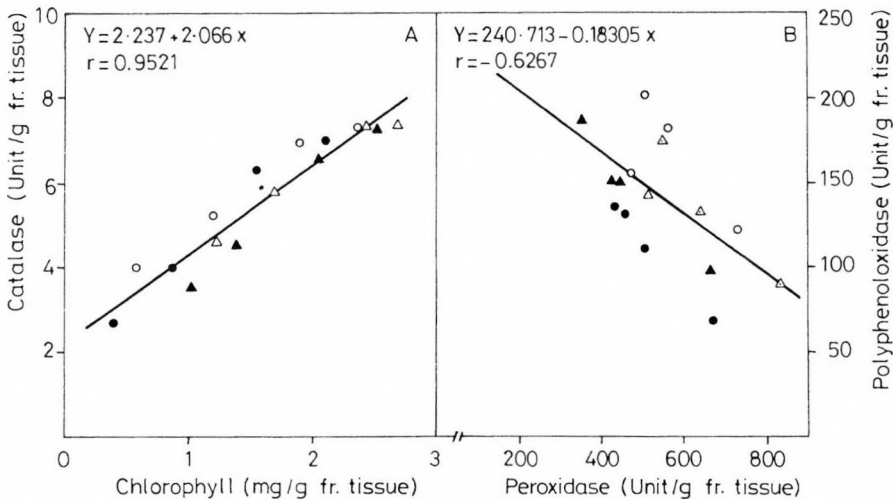
Fig. 4. Linear regression between chlorophyll content and catalase activity (A) and between peroxidase and polyphenoloxidase activities (B) of attached senescing rice leaves under control (o), NaCl-stressed (•), kinetin-treated (Δ) and kinetin-treated NaCl-stressed (▲) conditions

($r = -0.6267$, $n = 16$ at $p = 0.01$) was observed between the activities of peroxidase and polyphenoloxidase.

Discussion

The present investigation provides a study of the effect of salt-stress on the alterations of chlorophyll and protein levels and some enzyme activities in attached leaves during the process of senescence. The alterations common to the salt-stressed and non-stressed turgid leaves were due to the effect of senescence process per se while the differences in the levels of chlorophyll and protein contents and activities of enzymes at any particular time within the comparable leaf samples were due to stress effect alone.

During the process of senescence the metabolic status of the leaf is known by estimating mainly the loss of chlorophyll and protein (KAR and MISHRA 1976; SAHU and MISHRA 1987). As chlorophyll is an indicator of metabolic status of plants (YEO and FLOWERS 1983), any alteration in the chlorophyll content of leaves hampers the internal metabolic processes. In this experiment, salinization has been found to accelerate the loss of chlorophyll of attached senescing rice leaves which indicates that NaCl-stress hampers to a higher degree the metabolic status of the stressed leaves in comparison to that of the non-stressed leaves during senescence process. Retardation of chlorophyll loss by kinetin in senescing leaves has been reported by a number of workers (THIMANN 1985; SAHU and MISHRA 1987). The present work also confirms this role of kinetin in attached senescing leaves of rice, but at the same time indicates that the degree of retardation of chlorophyll loss by kinetin treatment is more pronounced in the stressed condition than in the non-stressed condition.

The other senescence parameter, loss of protein, had shown a similar trend as that of chlorophyll in both non-stressed and salt-stressed leaves. Kinetin treatment also arrested the decreasing trend of protein content to a higher degree in the NaCl-stressed than in the non-stressed leaves. The accelaration of protein loss by NaCl-stress and its alleviation by kinetin treatment in attached senescing rice leaves is similar to that in detached leaves reported earlier (SAHU and MISHRA 1987).

Catalase activity appears to consistently decrease during senescence regardless of the experimental models used (LAURIERE 1983). Salinity stress has been reported to both increase (STROGONOV 1964) and decrease (VASILE 1963) the catalase activity in leaves. In the present investigation, salt-stress accelerated the declining trend of this enzyme activity after 2 d while kinetin treatment retarded it. The decrease in catalase activity may

be related to the accumulation of the oxyradical, H_2O_2, a potent toxic agent to several key enzymes in leaves, which then accelerates oxidative processes associated with the senescence of leaves (BRENNAN and FRENKEL 1977; SAHU and MISHRA 1987; CHOUDHURI 1988) with further increase in leaves subjected to NaCl-stress. Treatment of kinetin, owing to this reason, delays the senescence process of both non-stressed and salt-stressed leaves. Further, as catalase activity has been taken as an indirect measure of respiratory rate (PAUL and MUKHERJI 1972), the lower level of this enzyme activity in the NaCl-stressed leaves may indicate decreased rate of respiration. However, the regression analysis showed that degree of leaf senescence as induced by NaCl-stress is closely correlated with the decrease in catalase activity.

Both increasing and decreasing tendencies of peroxidase and polyphenoloxidase activities during senescence of different plant species have been reported (KAR and MISHRA 1976; PATRA and MISHRA 1979; LAURIERE 1983). We have reported increased peroxidase activity only in salt-stressed senescing rice leaves in detached condition (SAHU and MISHRA 1987). In the present paper, we report the pattern of changes in both peroxidase and polyphenoloxidase activities in NaCl-stressed senescing rice leaves in attached condition. Our results show an increasing trend of peroxidase activity during the early phase of senescence (up to 4 d) with subsequent decreasing trend in the late senescent phase (up to 8 d) and in NaCl-stressed leaves the activity of this H_2O_2 utilizing enzyme comparatively became slower indicating a retarding effect of salt-stress. However, the declining trend of peroxidase activity in the late senescent phase, when catalase activity also decreased, may not be due to "induced protective reaction" as suggested by BIRECKA et al. (1979), but on the other hand, related to the accumulation of toxic H_2O_2 which, thus, enhances the senescence process with further enhancement under stressed condition. Contrary to the retarding effect of kinetin on the metabolic processes of leaf tissues, the present work shows an accelerating effect of kinetin on the changing pattern of peroxidase activity in both non-stressed and salt-stressed leaves, which needs further explanation, as the level of endogenous H_2O_2 in attached senescing stressed leaves is yet to be investigated for which further work is needed. In detached leaves, both increase in peroxidase activity and H_2O_2 level have been reported suggesting the limitation of the in vivo substrate of peroxidase and its inability to decompose H_2O_2 in senescing and stressed leaves (KAR and FEIERABEND 1984; SAHU and MISHRA 1987).

By visualizing the irregular drift in the activity of polyphenoloxidase alongwith that of peroxidase during the development and senescence of leaves of higher plants, it has been earlier suggested that the fluctuation of these enzyme activities in species-specific and cannot be taken as reliable indicator of senescence (PATRA and MISHRA 1979). In the present investigation on attached senescing leaves, polyphenoloxidase exhibited a reverse trend in its activity to that of peroxidase under both non-stressed and salt-stressed conditions. The lower level of this enzyme activity in the stressed leaves in comparison to the non-stressed leaves may be related to the accumulation of more toxic phenolic compounds which would enhance the senescence process faster in the stressed leaves. As per the regression analysis, the degree of leaf senescence is not correlated with the peroxidase and polyphenoloxidase which indicates that the pattern of these two enzyme activities in the attached stressed senescing leaves is irregular. However, a significant correlation between the peroxidase and polyphenoloxidase activities ($r = -0.6267$) suggests that when the former increases, the latter decreases and vice versa. This type of behaviour of these two oxidases may play a balancing role so that a steady accumulation of toxic phenolic compounds and oxyradicals are maintained that may accelerate the senescence process as induced by salinity stress.

In view of the above, it is clear that the pattern of enzyme activities in attached leaves is different from that of the detached leaves during the process of senescence as induced by salt-stress. In conclusion, our results suggest that salt-stress does not accelerate, and kinetin does not retard, all the biochemical processes associated with the senescence of attached leaves. Further, at least in rice, catalase activity can be taken as an indicator of attached leaf senescence under NaCl-stress, but not peroxidase or polyphenoloxidase activities.

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INFLUENCE OF DIFFERENT WAVE-LENGTH LASER LIGHTS ON THE CARBOHYDRATE METABOLISM IN GERMINATING MAIZE SEEDS

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The influence of 337.1 nm (0.1 J, IJ), 510 nm (0.5 J) and 632.8 nm (0.1 J, 1 J) wave-length laser lights on the carbohydrate metabolism of 1-6 day old germinating maize seeds was investigated. Seeds were treated in water-imbided state, then the glucose, soluble sugar and lactate content of the embryo and endosperm was determined daily for 6 days. The α-amylase and lactate-dehydrogenase (LDH) activities were measured.

Our results shows that the 337.1 nm and 510 nm laser lights changed the activity of .-amylase, one of the most important enzymes of the carbohydrate catabolism. The irradiation of 337.1 nm resulted in a higher activity compared with the control, while that of 510 nm brought about a lower one.

The glucose content of the embryo was lower than that of the control, at each treatment until the 4th day, while on the 5th day it became higher at the irradiation of 337.1 and 510 nm laser lights. In the endosperm, the 337.1 and 632.8 nm laser lights caused lower glucose content compared with that of the control only on the 3rd day, and the 510 nm resulted in the same outcome on the 4th day.

The 337.1 nm treatment caused different effect in the soluble sugar content. The treatment with 337.1 nm, 0.1 J energy resulted in a higher than normal, white 1 J in a lower soluble sugar level in the embryo. In the endosperm, applying the laser beam of both doses, soluble sugar content was higher than that of the control. As a result of 632.8 nm irradiating, the soluble sugar content measured in the embryo was lower than in control on the 4th day, while in the endosperm it was higher. The laser treatment with 632.8 nm caused higher lactate level on the 3rd day, and that of 510 nm on the 6th day. At this time the LDH activity decreased.

Introduction

The properties of laser beam differs from other electromagnetic waves: e.g. it processes a unique energy-material interaction. It has been shown that this interaction caused several biological effects (EUCHLER and LENZ 1977; BERKI et al. 1988; BURT et al. 1979; CSESZNOKOVÁ et al. 1982; PIKULEV et al. 1986; MARKOV et al. 1987; GREEN et al. 1987; KOVÁCS et al. 1982; VAN DER MEULEN and JUDY 1988; ANGELOV et al. 1988; TSANG et al. 1986; SZCSASZTLIVCEBA et al. 1979; DOBRIENKO et al. 1986; SCHEURLEIN and BRAS-

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LOVSKY 1985; FEHÉR 1982). Low-level laser light at various wavelengths and outputs had a favourable effect on germination (FEHÉR 1982), vegetative development and product of some agricultural plant (MARKOV et al. 1987; SZCSASZTLIVCEBA et al. 1979; DOBRIENKO et al. 1986). However, regarding its physiological and biochemical effects only a few reports have been published (PIKULEV et al. 1986; BERKI et al. 1984). Our aim was to investigate whether laser light has or has no an effect on the metabolic changes of the germination. In this report we demostrate the effect of He-Ne laser (λ = 632.8 nm), Nitrogen laser (λ = 337.1 nm) and dyelaser (λ = 510 nm) irradiations on the carbohydrate metabolism in germinating corn.

Material and Methods

Plant material: Hybrid corn seed (*Zea mays* L. convar. *saccharata*). After surface sterilization (3% hypochloric acid) the uniformsized seeds were soaked in sterile distilled water for 24 h at 20-22 °C, and the seeds were irradiated by the laser light separately.

Laser applications:

Equipment	Mode of operation	λ (nm)	P	Repetition frequency	J/seed	Radiation time (s)
MOM-77	permanent	632.8	5.6 mW	—	0.1 1	18 180
NDL-2 JPTE	impulse	510	0.232 mJ/imp.	10 Hz	0.5	210
NL-300 JPTE	impulse	337.1	2.5 mJ/imp.	10 Hz	0.1 1.0	4 40

Germination: The treated seeds were germinated for 6 days in dark at 20-22 °C in Petri dishes between double layer of filter papers moistened with steriled water.

Samples and detection: Seedlings were removed at daily intervals and embryo and endosperm were separated under visual control. Fifteen samples of tissue were used for each determination daily. Biochemical analysis of seedling parts was determined from dried material (48 h, 32 °C). The dried samples were homogenized in 5 ml 0.01 M phosphate buffer (pH = 6.7, 1 mM $CaCl_2$) at 4 °C using a power-driven potter (LR-40). Embryos were homogenized in the same buffer containing 0.1% Triton-X 100. The extracts were centrifuged at 3500 g for 20 min. Aliquots of this clear supernatants were used for all enzyme and metabolite determinations. Enzymatic methods were used to determine the content of glucose (WONG et al. 1981) and lactate (HADJIVASSILIOU and RIEDER 1968). Soluble carbohydrate (ASHWELL 1966), α-amylase (Phadebas-α-amylase-test) and lactate-dehydrogenase (Boeringer-test) were measured spectrophotometrically. For statistical calculation Student's t-test, and P values were applied.

Results

Effects of 337.1 nm laser light

During germination the α-amylase activity of the endosperm in the first 4 days increased slightly (Fig. 1). That seeds irradiated with 337.1 nm laser light, the α-amylase activity was higher than in the control seeds. The difference in the % of control values at 0.1 J treatment was 163%, and at 1 J treatment it was 185% measured on the 5th day.

The 337.1 nm laser light changed the glucose content of embryo and endosperm according to dose (Table 1). Highly elevated levels were found on the 3rd and 5th day of germination. Two-four days after the irradiation the glucose content in the treated embryos were lower than in the controls ($P < 0.05$), while on the 5th day these values became higher as a result of the 1 J energy treatment (Table 1a). 0.1 J treatment caused significantly higher soluble glucose content in the embryos only on the 5th day.

The glucose content of the endosperm in the treated seeds were lower than in the controls on the 3rd day of germination (Table 1b). The different irradiation energy in this case had no effect on the glucose level.
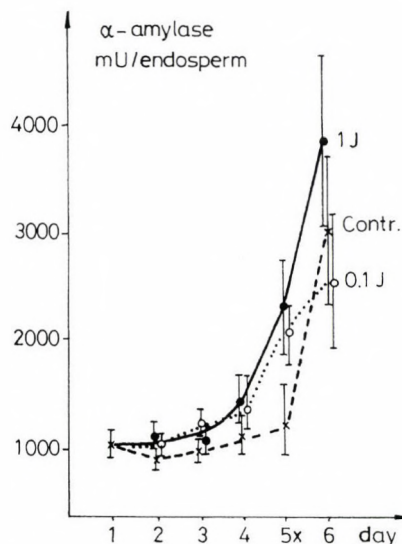
Fig. 1. Influence of 337.1 nm laser on the α-amylase activity of germinating maize seeds.

x: control	1247 ± 370	
0.1 J	2032 ± 324	$P < 0.001$
1 J	2310 ± 476	$P < 0.001$

Table 1

Influence of 337.1 nm laser light on the soluble glucose content of germinating maize seeds

a) Glucose μM/embryo				b) Glucose μM/endosperm.			
5th day	average ± s.d.	n	P<	3rd day	average ± s.d.	n	P<
K	24.73 ± 5.51	30	—	K	40.99 ± 5.67	30	—
0.1 J	39.12 ± 10.001	30	0.001	0.1 J	29.25 ± 3.38	30	0.001
1 J	32.78 ± 4.71	30	0.001	1 J	30.44 ± 7.52	30	0.001

During the mobilization of reserved nutrients, according to different function of seed parts, the total amount of soluble sugar gradually decreased in the endosperm, while in the embryo it increased. Our results show that the 337.1 nm laser light has changed the soluble sugar content in both the embryo and endosperm (Fig. 2).

In the embryo of untreated seedlings the sugar concentration has gradually increased from the 3rd day, while in the case of seeds treated with 0.1 J, it has changed according to maximum curve. The increase of soluble sugar content in seedlings treated with 1 J was lower than in the controls (Fig. 2a).

In endosperm the soluble sugar content was higher in the treated seedlings than in the untreated ones, and it seems to be dose dependent (Fig. 2b).
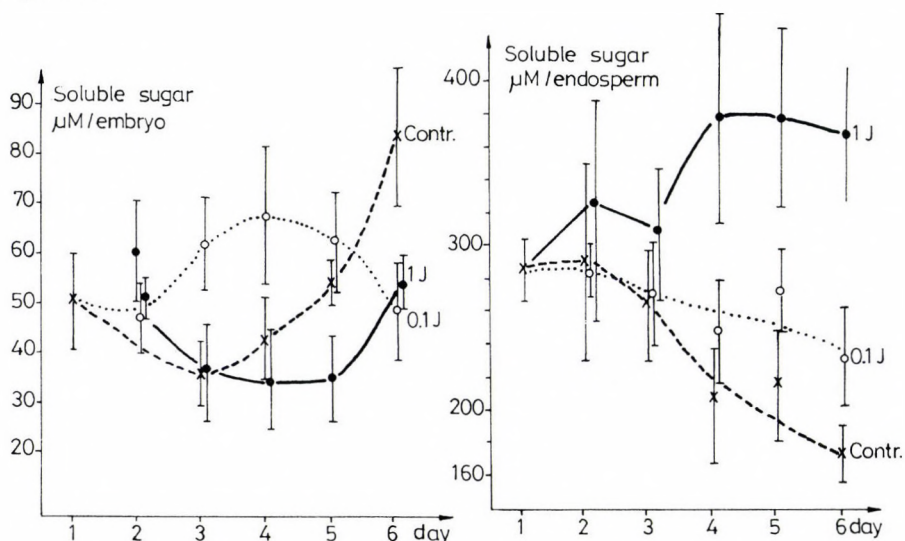
Fig. 2. Influence of 337.1 nm laser beam on the soluble sugar content of germinating maize seeds
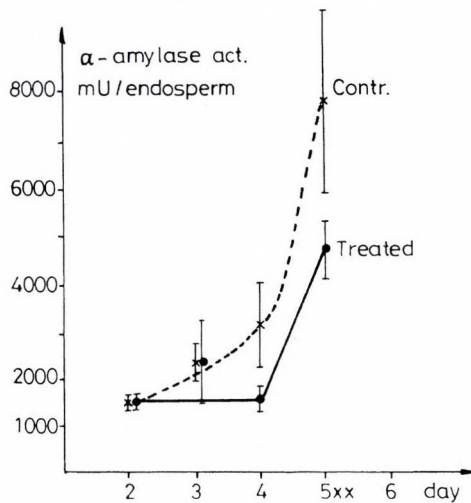
Fig. 3. Influence of 510 nm laser light on the α-amylase activity of germinating maize seeds.

x: control 3255 ± 975, treated 1777 ± 239, $P < 0.001$

xx: control 7911 ± 1968, treated 4952 ± 528, $P < 0.001$

The 0.1 J and 1 J energy of 337.1 nm laser light had no effect on the lactate content of germinating maize seeds.

Effects of 510 nm laser light

The α-amylase activity in the endosperm of cornseeds, irradiated with 510 nm laser light, was lower than in the control samples (Fig. 3). Significant difference was found on the 4th and 5th days of germination. Parallelly with the lower α-amylase activity after irradiation, we found a lower glucose

Table 2

Influence of 510 nm laser light on the glucose of germinating maize embryo and endosperm

Day	Average ± s.d. μM/embryo				Average ± s.d. μM/endosperm.			
	control	treated	n	P<	control	treated	n	P<
1.	10.21 ± 1.27	9.98 ± 2.41	20	—	10.50 ± 3.77	11.06 ± 4.99	20	—
3.	17.32 ± 4.06	12.25 ± 6.18	20	0.05	36.35 ± 14.50	34.18 ± 19.81	20	—
4.	42.57 ± 17.6	26.99 ± 4.42	20	0.05	76.59 ± 15.71	48.29 ± 15	20	0.001
5.	76.93 ± 9.19	124.8 ± 32.27	20	0.01	61.80 ± 30.61	70.81 ± 39.87	20	—

Table 3

Influence of 632.8 nm laser light on the glucose content of maize embryo

Day	Glucose average ± s.d. μM/embryo						
	control	0.1 J	n	P	1 J	n	P
1.	1.45 ± 0.28	0.75 ± 0.35	20	0.001	1.3 ± 0.34	20	—
2.	5.55 ± 1.24	4.35 ± 1.55	20	0.1	7.1 ± 0.8	20	0.01
3.	21.25 ± 4.73	15.05 ± 2.07	20	0.01	11.7 ± 2.03	20	0.001
4.	44.14 ± 15.23	30.05 ± 2.69	20	0.01	27.75 ± 5.42	20	0.01

content in the treated seedlings. The glucose content of embryo is shown in Table 2. The glucose level of embryo was lower than that of the control on the 2nd-4th days, while on the 5th day it was higher. The change of the glucose content in the irradiated seeds' endosperm was similar to that found in the embryo, but a significant difference was measured on the 4th day of germination (Table 2).
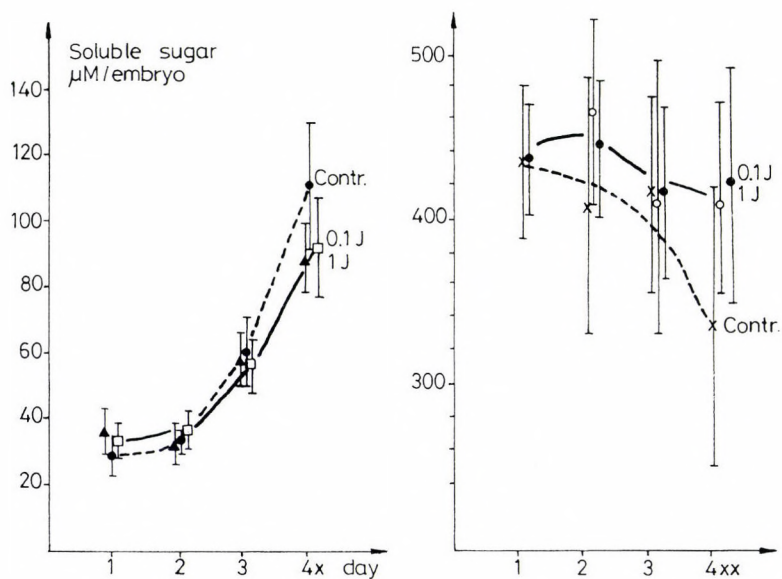
Fig. 4. Influence of 632.8 nm laser light on the soluble sugar content of germinating maize seeds.

x: control	110 ± 19 μM/embryo		xx: control	334 ± 87 μM/end.	
0.1 J	88 ± 13	$P < 0.01$	0.1 J	409 ± 55	$P < 0.05$
1 J	92 ± 18	$P < 0.1$	1 J	422 ± 75	$P < 0.05$

Table 4

Influence of 632.8 nm laser light LDH and lactate content of the germinating maize on the 3rd day

3rd day	LDH (U/L) average	Lactate (mM/L) average
contr.	50	0.70
0.1 J	0	0.90
1 J	10	0.87

After irradiating the seeds with 510 nm laser light, the rate of increase in the soluble sugar content in the embryo and its decrease in the endosperm did not differ significantly from the control.

This irradiation decreased the lactate content on the 6th day of germination. At that time, LDH activity was only one-fifth that of the untreated group (control: 31 U/L; treated: 6 U/L). Accordingly, lactate content was higher than in the control group (control: 1.38 mM/L; treated: 1.69 mM/L).

Effects of 632.8 nm laser light

No difference was measured concerning the α-amylase activity of endosperm between the treated and untreated groups. The 632.8 nm laser light caused a decreased glucose level of the treated seeds. The average values of glucose content in the embryo is shown in Table 3. In the endosperm we found a significantly lower ($P < 0.01$) glucose level with the irradiation of 1 J energy, on the 3rd day (control: 42.45 ± 9.61 µM/end.; 1 J: 30.95 ± 6.47 µM/end.). While the embryo developed, its soluble sugar content increased, while at the same time in the endosperm it has decreased. The He-Ne laser light treatment caused significant difference between the control and treated groups in the soluble sugar content of the seeds on the 4th day (Fig. 4).

As compared with the untreated group, we measured a lower sugar level in the embryo and a higher one in the endosperm on the 4th day. The two different doses of irradiation caused the same type of modification in the seed parts (Fig. 4).

A difference between the control and the treated groups was found in the lactate-IDH system on the 3rd day of germination (Table 4). We measured a low lactate level in relation to LDH activity in the embryo of the control group.

Discussion

We studied the influence of 337.1 nm, 510 nm and 632.8 nm laser light on the carbohydrate metabolism of 1-6 day germinating maize seeds. We have used laser equipment with different wave-lengths and energys according to the results of previous reports (BERTKI et al. 1984; YEW 1982). We chose metabolic parameters of carbohydrate metabolism which could fairly be measured in the investigated period of germination. The periodic change and order of magnitude of parameters defined in this way, were in accordance with data published (BEWLEY and BLACK 1985).

The applied laser irradiations caused significant changes in the carbohydrate metabolism of the germinating maize seeds. The main reserved nutrient in maize seeds is starch, therefore its utilization basically determines the development of the embryo. From the 2nd day of germination the utilization of stored nutrients gets started. The catabolism of the starch by amylase is well controlled. Both α- and β-amylase activity increase during germination, nine-tenth of total amylolytic activity is due to α-amylase. As an effect of 337.1 nm laser light the α-amylase activity was higher than the control value, while in case of 510 nm it was lower (Fig. 3). The irradiation with 632.8 nm did not modify the α-amylase activity. According to amylolytic activity the glucose content of the seeds grandually increases during germination. Each type of irradiation decreased the glucose utilization of the embryo until the 4th day. Applying 337.1 nm and 510 nm laser lights, the values we found were higher than the control ones on the 5th day (Tables 1a, 2, 3). The 337.1 nm and 632.8 nm irradiation caused lower glucose content in the endosperm compared with the control on the 3rd day (Table 1b).

Applying 510 nm laser light the glucose value in the endosperm corresponds to changed parallelly with that the embryo (Table 2). The various laser had different effect on the soluble sugar content. The 510 nm laser light did not modify the total sugar content of the seedlings. Applying 632.8 nm laser light, we found a lower soluble sugar level in the embryo and a higher one in the endosperm than in the control, on the 4th day. The irradiation with different energy of 337.1 nm wavelength laser light caused an opposite change in the embryo. The soluble sugar content increased in case of 0.1 J, while applying 1 J it decreased. In the endosperm both energies caused an increase in the soluble sugar content. The 337.1 nm irradiation
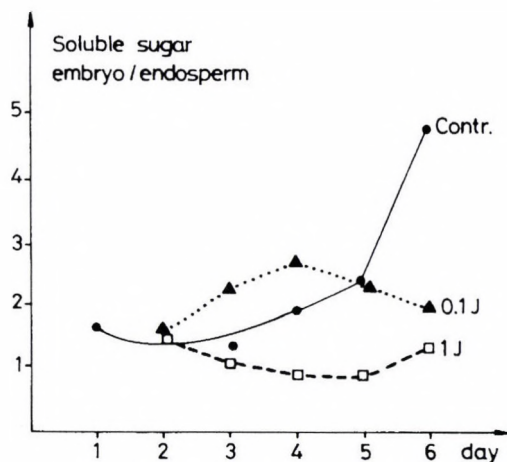
Fig. 5. Influence of 337.1 nm laser light on the soluble sugar content between the seed parts in germinating maize seeds

caused dose-dependent changes in the glucose content of the embryo and in the soluble sugar content of both seed parts (Table 1, Fig. 2).

The sugar levels in the embryo and the endosperm showed a different changing pattern according to doses of irradiation. The effect of UV laser light caused the most pronounced changes in the soluble sugar level (Fig. 5).

In the germinating seeds catabolic changes depend on anaerobic or aerobic pathways. Changes anaerob-aerob relations in the metabolism the LDH and lactate quantity were determined. The stored products of anaerobic respiration are ethanol and lactate. The level of lactic acid as a most characteristic anaerob product of the carbohydrate metabolism showed a significant increase at irradiation wavelength of 632.8 nm and 510 nm.

Differences arising after the applied laser light irradiations can be explained on one hand by the change of direction and/or intensity of metabolism; and on the other hand by the influence of transport processes and permeability relations between seed parts with different functions. These questions can be clarified by further investigations.

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AUXIN-, GIBBERELLIN- AND CYTOKININ-LIKE ACTIVITIES DURING GERMINATION OF MAIZE

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We have determined the changes of free auxin-, gibberellin- and cytokinin-like activities in dry, imbibed and germinated maize seeds and seedlings, in embryos and endosperms (24 h, 72 h) of maize (Zea mays L. convar. saccharata MV. Kerti édes F1.) seeds, using bioassays. We could not detect significant difference in gibberellin-like activities because of the low level, applied by biotest. We measured higher gibberellin content only in the endosperm of germinated seeds (72 h), which shows close correlation with increasing of α-amylase enzyme activity.

Profiles of the auxin- and cytokinin-like activities on paper chromatograms are shown and their maximum and minimum type tendency during the investigated period are discussed in this paper.

Introduction

Most of the evidence of the roles of plant growth regulators in the germinative processes is based on the effects of external application of compounds to the seeds.

Investigation of endogenous plant growth regulators is not easy, because of the low levels of regulating substances present and the complexity of the sample extracted. Even the modern techniques, such as gas chromatography, HPLC or immunoassays can have hidden errors and can result in fals values. Besides, only a few laboratories apply them for routine analysis. Bioassays, which in turn have been used for many years, can, however with proper precautions, serve as important tools both in the detection of compounds or derivatives showing biological activity or as biospecific detectors following chromatography but their sensitivity are various (EPEL et al. 1987).

Abbreviations: IAA = indole-3-acetic acid; GA_3 = gibberellic acid; ABA = abscisic acid; BAP = 6-benzylaminopurine; HPLC = high performance liquid chromatography

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We have determined simultaneously the changes of the free auxin, gibberellin and cytokinin levels during imbibition and germination of sugar maize (Zea mays L.), using bioassays. These results together with other investigations of metabolism can help to follow the process of germination.

Material and Method

Plant material: Hybrid maize seeds and seedlings (Zea mays L. convar. saccharata, MV. Kerti édes F1). The mature dry seeds (40 g = 150 ± 3 pieces) were imbided for 24 h in dest. water or germinated for 72 h in dark at 24-25 ^{0}C, on a moistened filter paper. In this time (72 h) practically all seeds were germinated, the length of radicle between 0-80 mm (most of them 10-30 mm). After imbibition or germination the embryo and endosperm were separated and collected for extraction.

Extraction and purification (VLASOV et al. (1979): The grinded dry seeds and collected embryos and endosperms were homogenized in and re-extracted with 70% ice-cold ethanol (12 h, 2 h, 2 h).

The combined ethanolic extracts of each sample were reduced to an aqueous residue by evaporating the ethanol in vacuo. Each extract was divided into three equal parts — to determine (1) auxins; (2) gibberellins; (3) cytokinins — and were frozen. After melt at 4 ^{0}C each sample was centrifuged (10 000 rpm/10 min) and the supernatants were adjusted to pH 2.5 with dilute HCl. The first parts of each sample were fractionated into cold diethyl ether; the second parts into ethyl-acetate three times and the organic fractions were collected. The ether and ethyl-acetate were removed in an evaporator and the residues dissolved in 2 ml 96% ethanol. The third parts of samples were passed through a Dowex 50 W x 8 cation exchange resin (H^+ from). Cytokinin-like compounds were eluted from the column with 200 ml 4 N NH_4OH (VAN STADEN 1976). The eluates were evaporated in vacuo and residues taken up in 2 ml 96% ethanol. Ethanolic samples were loaded onto Whatman No. 1 chromatography papers and separated with isopropanol: 25% NH_4OH : water (10:1:1 v/v). Under the same conditions of chromatography the authentic standards and their R_f are shown on Table 1.

Bioassays: The chromatograms were divided into 9 equal strips and these strips were then assayed for auxin with coleoptile straight growth bioassay (NITSCH and NITSCH 1956), wheat seeds, Triticum vulgare L. var. Albidum-43; for gibberellin with bioassay: hypocotil growth of lettuce seedlings (FRANKLAND and WAREING 1962), lettuce seeds, Lactuca sativa L. var. berlinskii; and for cytokinin with Amaranthus betacyanin (BIDDINGTON and THOMAS 1973) bioassay (Amaranthus caudatus L.).

Results and Discussion

While the germinating seed is utilizating its storage reserves, other changes are also occur to its content of important chemical substances — the growth regulators or hormones, auxin, gibberellins, cytokinins and ABA. These substances are thought to play important roles in the regulation of certain aspects of seed growth and development as well as in other physiological phenomena.

Different articles are dealing with the levels of plant growth regulators during seed germination (TILLBERG 1977; TILLBERG et al. 1981; SHYMALI SAHA 1984; SMITH and VAN STADEN 1978). They investigated different plants, used different methods and determined ususally only one type regulator compound. Our aim was to measure their level at the beginning of seed germination. We used dry, imbibed and germinated maize seeds and seedlings. At the same time we measured in both embryo and endosperm the auxin-, gibberellin- and cytokinin-like activities using different bioassays and wanted to determine their tendency in time.

The role of gibberellic acid in the formation and secretion of a great number of enzymes is the most throughly documented case of an endogenously produced hormone, which controls seed metabolism. In many cases the gibberellins are present in conjugated forms in seeds. Most of the research on the effect of gibberellins on the metabolism during germination has been concentrated on barley seeds (MAYER and SHAIN 1974). Gibberellic acid-like substances formed in barley scutella during the first two days of germination move out of embryo into the endosperm and aleuron layer. There is no doubt that gibberellins in some way control the de novo synthesis of α-amylase in the aleuron (GUBLER et al. 1987) however the production of α-amylase is not the primary response to gibberellic acid and occurs after a time lag in barley. In our samples extracted from maize we could not detect difference in gibberellin-like activities between embryo and endosperm. We measured activities (in % of control around 130-150%) with lettuce hypocotil growth bioassay in two strips (R_f 0-0.3 and 0.6-0.9). These gibberellin-like activities are very low, comparing with our GA_3 standard which in 2.5 mg/l concentration caused more than 400% stimulation in the same bioassay. Our result is not unexpected. In the acidic ethyl-acetate fractions we could measure the activities of endogenous free gibberellins. We measured higher activities only in the germnated seeds (72 h, endosp.). This shows close correlation with increase of α-amylase enzyme activity in maize.

Seeds of several species, e.g. maize, contain auxin conjugates, many of which furnish IAA upon hydrolytic cleavage. Thus, IAA from the conjugates in the endosperm of germinated maize (and probably other cereals) is translocated to the coleoptile tip of the growing seedlings (BEWLEY and BLACK 1985). TILLBERG (1977) determined the content of IAA in dry and germinating maize seeds. The highest level of IAA was found during that period, when the radicles began to emerge from the seed coat.
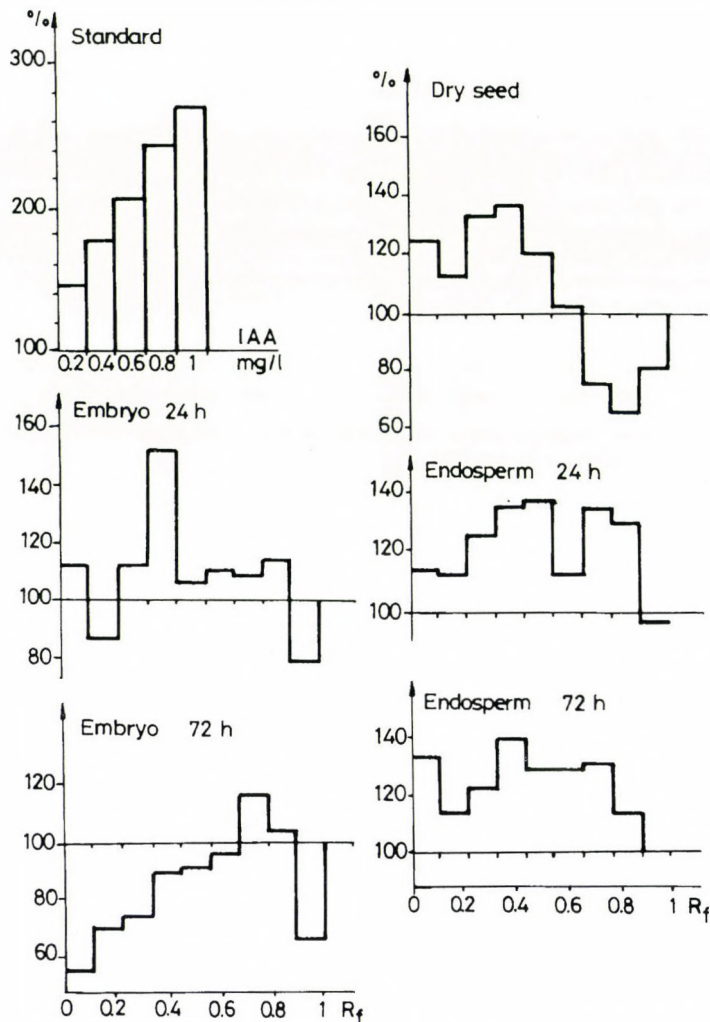
Fig. 1. Paper chromatogram profiles of the auxin-like activity in extracts from dry seeds; embryos and endosperms of germinating maize seeds (24 h, 72 h). Elongation of coleoptil segments in percent of control

We measured the same maximum-type change whith bioassay in seed parts (Fig. 1). We found the highest auxin-like activity in embryo after 24 h imbibition. This peak looks clean IAA as regards R_f (Table 1). At the same time we detected wider ranges in the endosperm. This shows the presence of other indole-compounds in our samples. The auxin-like activity in embryo is much more higher than in endosperm considered the rate of fresh weight. In this period of germination the endosperm is still almost four times bigger

Table 1

R_f values of standard growth regulators on paper chromatogram

Standards	R_f
IAA	0.36
GA_3	0.71
Zeatin	0.80
Kinetin	0.85
ABA	0.75

Table 2

Endosperm/embryo rate of fresh weight, during germination of maize

Germ. time	Rate of fresh weight endosp./embryo
24 h	3.8 ± 0.05
72 h	2.0 ± 0.15

(Table 2). In dry seeds we determined not only auxin activity (R_f 0.2-0.4) but inhibitor activity as well (R_f 0.7-0.9). Most probably this inhibitor is the abscisic acid (ABA) (Table 1), which influence both dormancy and post-germinative growth. During germination time its level declined fast. Our results provide further evidence that auxin-like activities occur in dry seeds in extractable free forms or easily release from conjugated forms. This fact may indicate that auxin can be important in the germination processes.

Reproductive structures are not only rich in endogenous cytokinin but also contain a great diversity of these compounds. Many investigations have yielded chromatographic, ultra-violet spectral and enzymic evidence which indicate that zeatin and its derivatives are present in seeds during germination. Compared with developing seeds the mature dry seed contains low levels endogenous cytokinins. These levels may remain low (SYMALI SAHA et al. 1984) or decrease during germination (SMITH and VAN STADEN 1978; THOMAS and KHAN 1976) or they may increase during this period. SMITH and VAN STADEN (1978) measured the changes in endogenous cytokinins during the germination of non-dormant kernels of Zea mays. In mature seeds were identified cyto-
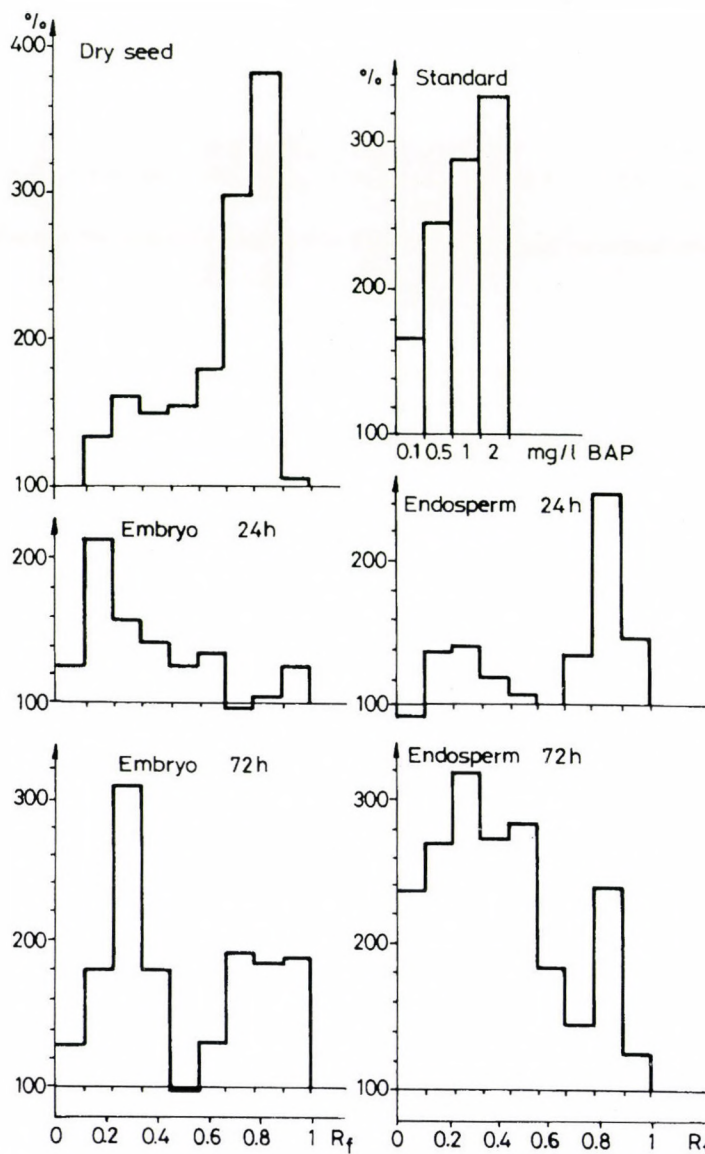
Fig. 2. Paper chromatogram profiles of the cytokinin-like activity in extracts from dry seeds; embryos and endosperms of germinating maize seeds (24 h, 72 h). The results in percent of control

kinins. They concluded that endogenous cytokinins in maize appear to play a prominent role during germination.

We determined cytokinin-like activities with Amaranthus betacyanin bioassay (Fig. 2). Qualitative and quantitative changes were observed in

both embryo and endosperm. The lowest level was detected in the samples after 24 h imbibition. In dry seeds we measured very high activity between R_f 0.7-0.9. This range can be zeatin, respectively (Table 1). Within this zone we found smaller activities in the other samples. It seems, that cytokinin-like activity changed with minimum-type curve in time of germination. Later (72 h) other peaks appeared on the chromatogram-profiles (R_f 0.1-0.4), which can represent other forms of cytokinins.

The whole topic of plant hormones in relation to seed dormancy and germination has been reviewed extensively on numerous occasions (KHAN et al. 1978; KHAN 1982 and references therein). The levels of plant growth regulators in seeds of different species were found to vary greatly. There is ample evidence that the release of seeds from dormancy is regulated by interactions between abscisic acid, gibberellins and cytokinins (MAYER and SHAIN 1974), and auxin plays a minor role in these processes. However auxin, present in germinating seeds is important at least for the cell division and cell growth occurring in the growing embryo. The hormonal requirements for development and germination of a seed appear to be different. Although in most case gibberellins play a primary role, cytokinins have promoting role during germination, but auxins and inhibitors influence the development of plant too. Besides, the environmental factors appear to affect the natural balance of hormones. Results of our investigations, the balance and change of free plant growth regulators in germinating maize seeds, help to explain our other measurements of metabolism during the same period in the same seed material.

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A NEW PROCEDURE FOR CHROMATOGRAPHIC PARTITIONING OF CARTHAMINE INTO PLURAL FORMS

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Investigations on the chromatographic partitioning of carthamine were conducted by using a preparative chromatographic method on insoluble polyvinyl pyrrolidone (PVP). A purified sample of carthmanine-dye, which migrates homologously on cellulose and silica gel thin-layer plates, were separated clearly on the PVP column developed with mixtures of acetone/methanol and acetone/ethanol. The two resolved bands were collected and purified severally through column chromatographies on synthetic polymers. The data from spectrometric analyses of the purified carthamines indicated that they are composed of closely related chemical structures.

Introduction

Carthamine is a representative dye-stuff of dyer's saffron (Carthamus tinctorius L.) used for dyeing cotton textiles and for producing cosmetic goods (SAITO 1989; SAITO et al. 1989; KANEHIRA et al. 1990; SAITO 1991). Recently, its fine red coluration has increasingly been recommended to apply as a food colourant (SAITO and FUKUSHIMA 1987; SAITO 1990; SAITO et al. 1993a, b, c). The chromatographically purified colouring matter exhibits three main UV/VIS absorption peaks at 521 (peak I), 330 (peak II) and 244 nm (peak III) in aqueous methanol. It migrates usually as a single spot on cellulose and silica gel TIC plates with specific R_f-values. These apparent chromatographic and spectroscopic behaviours is apt to mislead us to be

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Abbreviations: PVP = insoluble polyvinyl pyrrolidone; ODS = octadecyl silane; TMS = =trimethyl silane; TLC = thin-layer chromatography; CC = column chromatography; HPLC = high performance liquid chromatography; UV = ultraviolet light; VIS = visible light; IR = infrared light; ^{1}H-NMR = proton nuclear magnetic resonance.

R: p-hydroxycinnamoyl
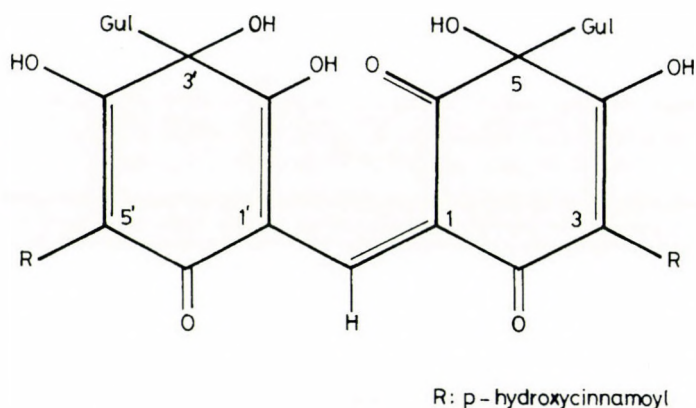
Fig. 1. Structure of carthamine

homologous component. However, its chemical structure calls teh external homogeneity in question if the dye is unmixed constituent: it comprises two asymmetric carbon atoms at 5 and 3' positions in the mother skeleton (Fig. 1) (TAKAHASHI et al. 1982).

Distinct separation and partial characterization of carthemine were the main object of this study, by which we aimed to justify the pertinence of our supposition on the chemical complexities of carthamine-dye. And, at the same time, we planned to find out a new efficient column packing for generally usable to partition homologous metabolites.

Material and Methods

Materials

The plant material was collected from the freshly opened flowering heads of dyer's saffron, which was cultivated carefully on our field for about three months. PVP was purchased from Sigma Chemical (St. Luis, MO, USA). Toyo Pearl HW-40f was purchased from Toyo Soda Kogyo (Tokyo, Japan). Sephadex LH-20 was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Chromatorex ODS was supplied by Fuji-Davison Chemical (Kasugai, Japan). Fract Gel PG M-2000, exlyene, benzene, dyethly ether, acetone, methanol and ethanol were purchased from Wako Pure Chemical (Osaka, Japan). Avicel cellulose was a product of Asahi Kasei Kogyo (Tokyo, Japan). Other chemicals used were obtained from several suppliers.

Preparation of processed flowers

Fresh flowers in distilled water (2 ml/g fresh weight) were ground into small pieces in a porcelain mortar with a pestle, covered with a silon film and left over-night at room temperature to develop red colour. The resulting flower pastes were suspended in appropriate amounts of distilled water, transferred to teflon tubes, centrifuged and the supernatant was sucked out. The pellet was suspended in distilled water and centrifuged again. The water-wahing was repeated twice or three times, and the cleaned pellet used to the following extraction process.

Extraction and partial purification of carthamine

The flower pastes were suspended in 10 ml/g fresh weight of 0.5% (w/v) K_2CO_3, stirred with a glass bar, poured into teflon tubes, centrifuged and the supernatant retained, K_2CO_3 extraction was done further twice and combined extracts were acidified to pH 4-5 by the addition of solid citric acid. To the acid solution, 0.5-1 g Avicel cellulose was suspended and stirred on amagnetic strirrer for several minutes. The reddish orange Avicel was washed three times with distilled water through centrifugation. The partially purified carthamine on Avicel cellulose was recovered by aqueous acetone (60%, v/v) and stored at -20 ^{0}C after being condensed under reduced pressure.

Chromatographic purification of carthamine

Carthamine sample was loaded on a top of Avicel cellulose column and chromatographed in n-butanol saturated with water. Carthamine eluate was evaporated, then charged again to a new Avicel column and CC was done in 65% (v/v) methanol. The carthamine eluate was rechromatographed on Toyo Pearl HW-40f in 70% (v/v) methanol and then on Sephadex LH-20 in 70% (v/v) acetone. A single carthamine band eluted from the column was collected and evaporated to dryness.

Partitioning of carthamine on a PVP column

For the chromatographic partitioning of carthamine, three solvents were exclusively used by various compositions as follows (v/v = experiment No.): methanol/water (40 : 60 = 1, 60 : 40 = 2), acetone/water (40 : 60 = 3, 50 : 50 = 4), acetone/methanol (40 : 1 = 5, 40 : 2 = 6, 40 : 3 = 7, 40 : 4 = 8, 40 : 5 = 9), acetone/ethanol (40 : 3 = 10, 40 : 4 = 11). PVP in glass columns (8 x 20 mm each) was pre-washed with 40 ml each test solvent as described above. Pure carthamine sample (437 µg) was dissolved in test solvents (1 ml each) and charged carefully onto the column top, which was developed descendingly in developing solvent desired. When solvent front reached at 10 cm below the column top, chromatogram was examined closely: migration distance and colouration were recorded.

Purification of separated carthamine

The resolved charthamine bands were collected and evapolated to dryness, then each carthamine band was CC. In this process, Toyo Pearl HW-40f, Sephadex LH-20 and Chromatorex ODS were used as column packings. 60% (v/v) methanol, distilled water and 70% (v/v) acetone were the developing solvents, respectively.

Spectroscopic registration of purified carthamines

UV/VIS spectra were recorded in 65% (v/v) methanol with a Shimadzu, model MPS-2000 spectrophotometer. IR spectra were registered in KBr disks with a Shimadzu, model IR-400 spectrometer. ^{1}H-NMR spectra were measured in mixtures of pyridine-d_5-methanol-d_4 (95 : 5, v/v) with a JEOL JNM FX-400. TMS was added as internal standard.

Results

Composition of solvent systems and migration pattern of carthamine

Figure 2 shows a typical result from comparison of solvent composition and carthamine migration profiles on PVP columns. No sign of carthamine separation is detectable in methanol/water (1 and 2). Aqueous methanol is not suitable for partitioning carthamine. On replacing of methanol with acetone, the migration pattern changes altogether: carthamine samples is separated clearly into two resolved bands (3-4). To obtain more affirmative consequence, new developing solvent systems, acetone/methanol and acetone/ethanol mixtures were prepared. The chromatograms are depicted in plates 5-9 and 10-11. Clear-cut separation of carthamine can be achieved in both solvents. The two red bands were collected separately and purified through CC.

UV/VIS spectral pattern of resolved carthamines

UV/VUS spectra of two carthamines were recorded in 65% (v/v) methanol from 200 to 600 nm. The data are presented in Fig. 3, showing that they
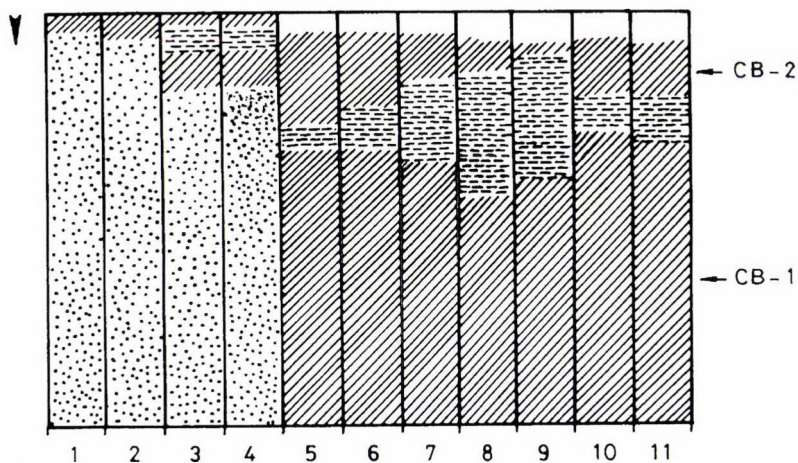
Fig. 2. Partition profiles of carthamine on PVP columns. An aliquot of carthamine sample was charged on the top of each PVP column (8 x 20 mm) and chromatographed in given compositions of desired solvents. Upon reaching the solvent front at 10 cm below the column top, the partition patterns were recorded as shown in the figures. The separated red bands were tentatively referred as CB-1 (fast-eluted band) and CB-2 (slow-eluted band). The number given at the bottom of each column indicates experimental No. For details solvents and their compositions, see Materials and Methods. ////: deep red, ----: light red, ::::: orange yellow. The arrow shows the direction of chromatography
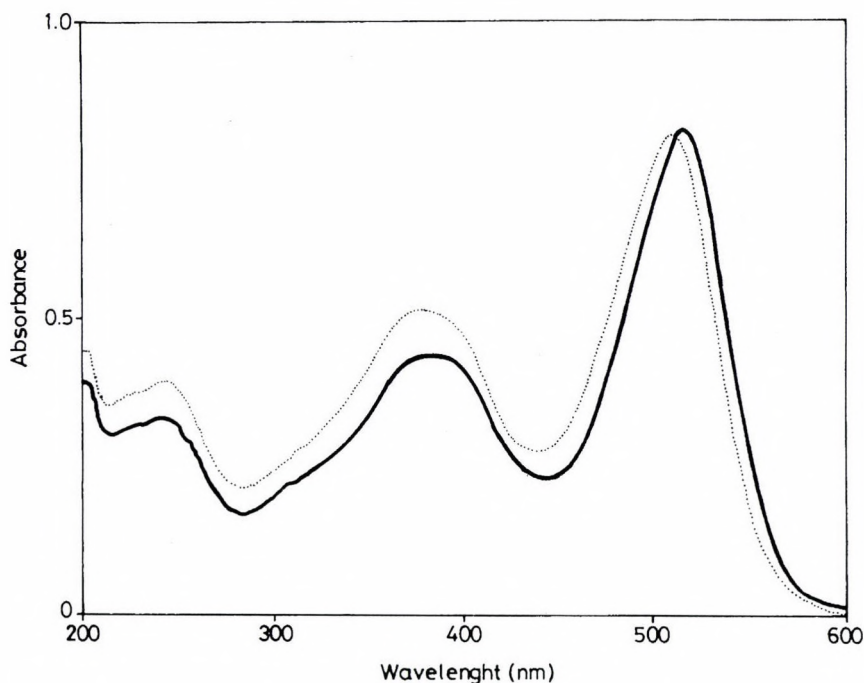
Fig. 3. UV/VIS absorbance spectra of separated carthamines. Aliquots of CB-1 and CB-2 were dissolved in 65% (v/v) methanol, and UV/VIS spectra monitored with a Shimadzu spectrophotometer, model MPS-2000. —: CB-1, ···: CB-2
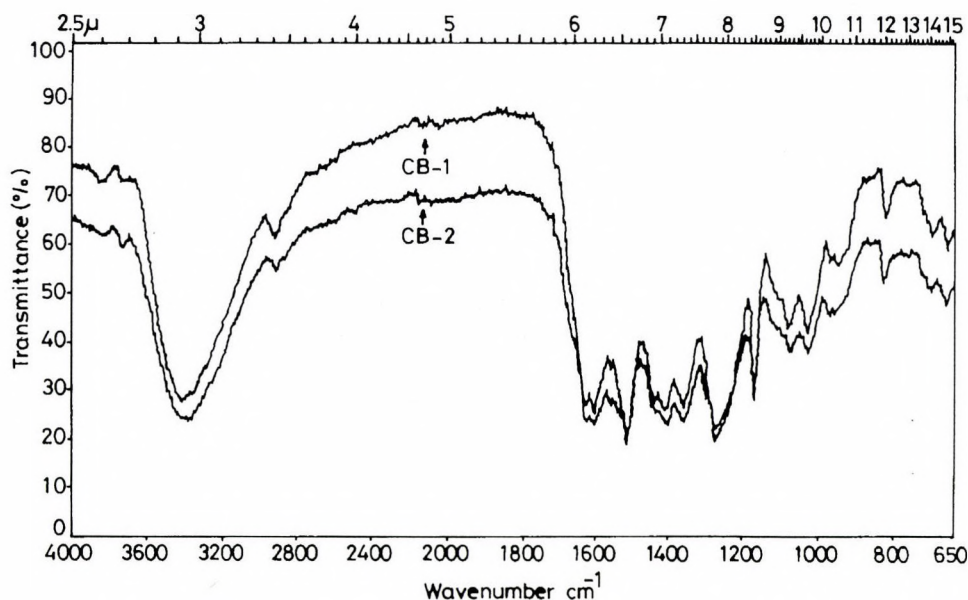
Fig. 4. IR spectra of separated carthamines
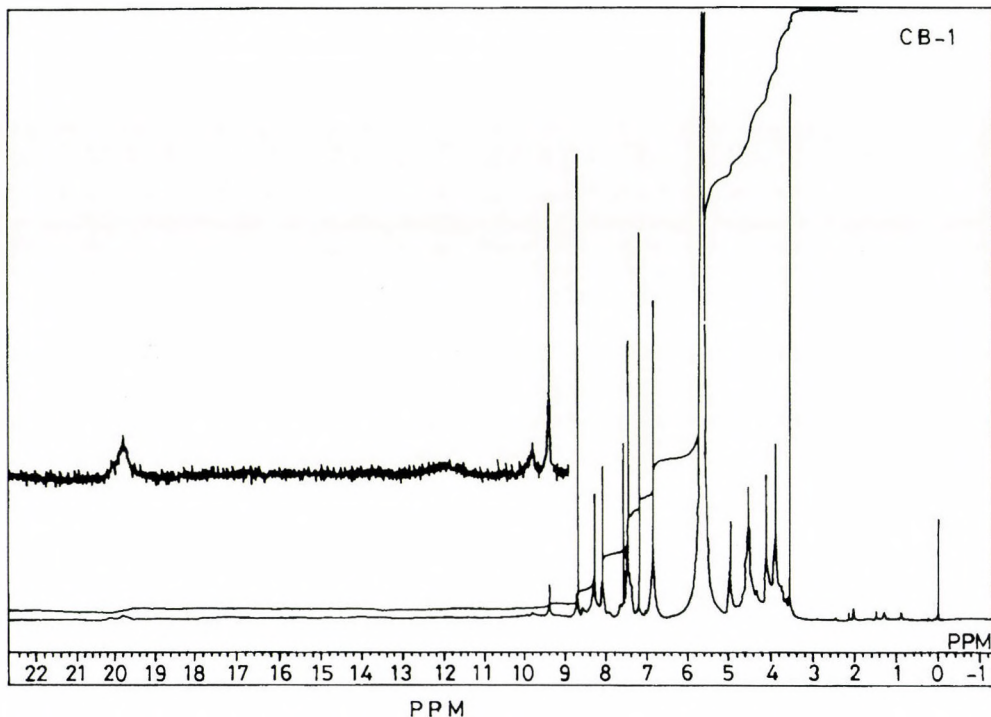
Fig. 5. ^{1}H-NMR spectra of separated carthemines. Both samples were measured in the mixtures of pyridine-d_5/methanol-d_4 (95 : 5, v/v). TMS was added as internal standard

follow quite similar curves. Three UC/VIS absorbance peaks are seen in both carthamines: 520.0/519.2 (peak I), 382.6/382.0 (peak II), 243.8/244.0 nm (peak III). These spectral peaks are considered to assign to cinnamoyl residue, benzoyl systems and olefinic bonds of carthamines.

IR spectral pattern of resolved carthamines

The IR spectra of the resolved carthamines are presented in Fig. 4. Phenolic and alcoholic hydroxyls, streching vibration of carbon-hydrogen from aromatic ring systems, trans-olefin and asymmetric streching vibration from acyclic ethers are all reciprocal with each other in finer details.

^{1}H-NMR spectral pattern of resolved carthamines

The rough assigment of protons to specific signals was based on comparison of the observed δ (ppm) for internal standard, TMS (Fig. 5A, B).
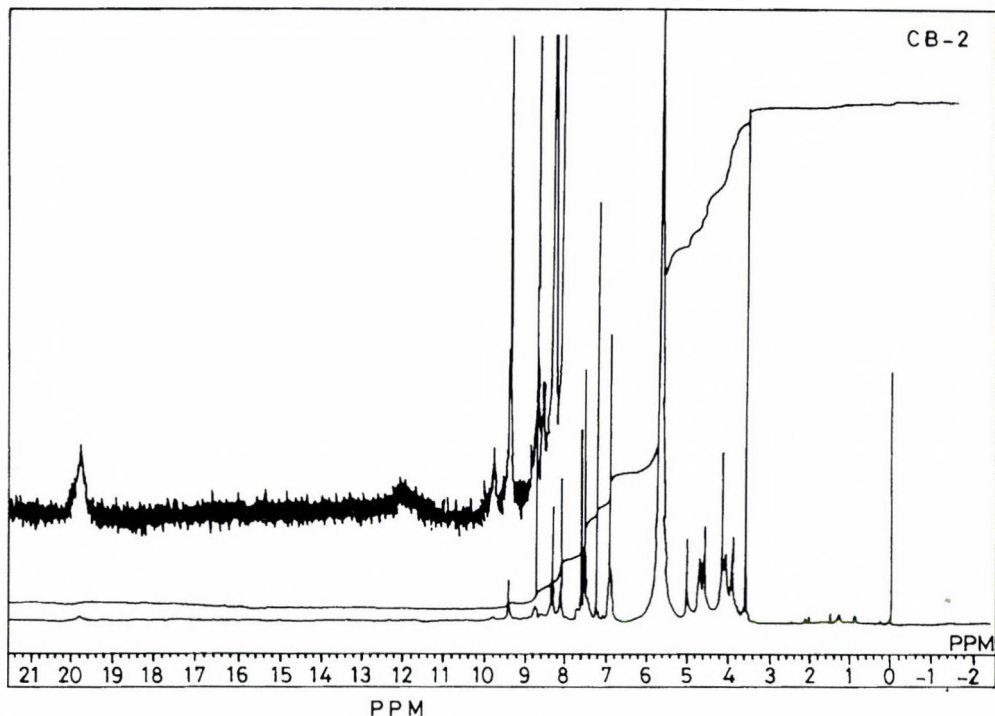
Fig. 5b.

Two carthamines exhibit peaks at 9.3 ppm and about 7.3-7.4 ppm result from p-hydroxycinnamoyl moieties, a signal at 9.3 ppm which is assignable as hydrogen atom from one olefin bond, two double signals at 6.80 ppm due to protons on aromatic rings. These data coincide virtually with each other, supporting above results that the molecular skeleton of both carthamines are constructed by analogous frames.

Discussion

A carthamine sample loaded onto a column top of PVP was developed ascendingly in aqueous mixtures of acetone/methanol or of acetone/ethanol. During the chromatographic process, it was dissolvaed down in the synthetic polymer dividing into two red strips. Here, the resolved bands were referred tentatively as carthamine band-1 (fast migration band) and carthamine band-2 (slow migration band). After recovery of these red bands, they were several-

ly purified on three different column packings and subjected to the spectroscopic analyses (Figs 3--5). The data indicate that two carthamines are constructed by very closely related chemical structures: UV/VIS absorbance spectra have many points of resemblance, IR spectra are reciprocal with each other in finer details, the signals assigned from the ^{1}H-NMR spectral analysis are almost coincident with one another, especially the δ(ppm) values at many positions on the mother skeleton (see Fig. 5A, B).

In this works, there has been clarified for the first time that plural forms of carthamine are co-existent in the floral tissues of dyer's saffron. Carthamine is mixtures of analogous semi-qunoidal chalcone glycosides. To partition natural mixtures with such infinitesmal structural differences, CC on PVP is very useful technique. Two carthamines thus separated will be applicable to reveal the chemical constitutions and/or to study their specific tinctorial properties.

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PRELIMINARY STUDIES OF POSSIBLE Ni-HYPERACCUMULATORS IN THE SERPENTINE FLORA OF CUBA. II.

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The authors — continuing their studies on the serpentinophilous plants of Cuba for finding further new Ni-hyperaccumulators — tested 158 species belonging to 57 families. All the studied plants were collected on soils derived from ultramafic (serpentine) rocks. They confirm all the formerly postulated statements (BORHIDI et al. 1993) about the taxonomic background of Ni-hyperaccumulation and contribute some new conclusions.

Introduction

Together with the former study 386 species belonging to 60 families and 148 genera has been tested for Ni-hyperaccumulation with a qualitative-semi-quantitative method using Waltham N-testing indicator papers (see BORHIDI et al. 1993). For this study the Herbarium materials of the Botanical Department of the Janus Pannonius University (JPU) was used, collected by the second author and his Cuban collaborators (R. CAPOTE, M. FERNANDEZ, O. MUÑIZ, R. OVIEDO, M. A. VALES) during their field works published in the "Phytogeography and Vegetation Ecology of Cuba". The collected materials originates from the ultramafic (serpentine) areas of Cajalbana and Sierra del Rosario (West-Cuba), Loma de Coca, Canasi, Corral Nuevo, Camarioca, Cerro Pelo Malo, Presa de Agabama, Arroyo Blanco de Jatibonico (Central Cuba), and Holguin, Sierra de Nipe, Sierra de Cristal, Sierra de Moa, Cuchillas de Toa y Baracoa, Peladeros de Jauco (East-Cuba).

Synchronously with this work a great number of these species was submitted to a quantitative analysis by Roger REEVES (Massey University, New Zealand) and the results of the qualitative testing for pre-selection proved to be completely in accordance with those of quantitative analysis. So, with the qualitative testing has been justified as semi-quantitative probes as well. Four grades of the colour-reaction could be determined:

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no or very pale coloration	= – or +	= 0—100 ppm
pale coloration	= ++	= 80—800 ppm
good coloration	= +++	= 800—8000 ppm
strong coloration	= ++++	= 7000—70 000 ppm

The results of the qualitative testing are listed in Table 1.

Table 1

Qualitative analysis of possible Ni-hyperaccumulators of the flora of Cuba. II

1. Fam.:	Anemiaceae	
	Anemia cajalbanica Borhidi	-
2. Fam.:	Annonaceae	
	Annona cristalensis (Alain) Borhidi et Moncada	-
3. Fam.:	Apocynaceae	
	Cameraria arborea Bisse	-
	C. latifolia L.	-
	Neobracea valenzuelana (A. Rich.) Urb.	-
	Rauvolfia salicifolia Griseb.	-
4. Fam.:	Aquifoliaceae	
	Ilex repandoides Loes.	-
5. Fam.:	Araliaceae	
	Dendropanax nervosus (Urb. et Ekm.) A.C.Sm.	-
6. Fam.:	Asteraceae	
	Baccharis shaferi Britt.	-
	Chaptalia shaferi Britt.	-
	Erigeron cuneifolius DC.	+
	Eupatorium grandiceps Wr. in Sauv.	++++
	E. grisebachianum Alain	-
	E. hypoleucum Griseb.	-
	E. lantanifolium Griseb.	-
	E. nipense B.L. Robins.	-
	E. rhexioides B.L. Robins.	-
	Gochnatia cubensis (Carab.) Jervis et Alain	-
	G. parvifolia (Britt.) Jervis et Alain	-
	G. shaferi (Britt.) Jervis et Alain	-
	Gundlachia apiculata Britt. et Blake	-
	G. lindeniana (A. Rich.) Urb.	-
	Harnackia bisecta Urb.	-
	Heptanthus ranunculoides Griseb.	-
	Lescaillea equisetiformis Griseb.	-
	Pentacalia almironcillo (Maza) Borhidi	++
	P. moaensis (Alain) Borhidi	+++
	P. trichotoma (Greenm.) Borhidi	++++
	Phania cajalbanica Borhidi et Muñiz	+
	Piptocoma ekmanii Alain	-
	Senecio azulensis Alain	++++
	S. plumbeus Griseb.	++++ (Nipe)
	S. plumbeus Griseb.	++++ (PR)

Table 1 (cont.)

	S. polyphlebius Griseb.	+
	Vernonia angustissima Wr. ex. Ekm.	-
	V. calophylla Gleas.	-
	V. hieracioides Griseb.	-
7. Fam.:	Bignoniaceae	
	Spirotecoma spiralis (Wr. ex Griseb.) Pichon	-
	Tabebuia acunae Borhidi	-
	T. cuneifolia Urb.	-
	T. lepidota (HBK) Britt.	-
	T. linearis Alain	-
	T. simplicifolia Carab. ex Alain	-
8. Fam.:	Boraginaceae	
	Bourreria puciflora D.E. Schulz	-
	Cordia pedunculosa Wr. ex Griseb.	-
	Heliotropium humifusum HBK	-
9. Fam.:	Burseraceae	
	Commiphora inaguensis (Britt.) Moncada	-
10. Fam.:	Buxaceae	
	Buxus aneura Urb.	-
	B. wrightii Muell. Arg.	-
11. Fam.:	Caesalpiniaceae	
	Caesalpinia nipensis Urb.	-
	Cassia bucherae M. Vict.	-
12. Fam.:	Celastraceae	
	Cassine nipensis (Bisse) Borhidi	-
	Maytenus parvifolia (A. Rich.) Mory	-
	M. revoluta Alain	-
13. Fam.:	Clusiaceae	
	Clusia nipensis Borhidi	-
	Rheedia fruticosa Wr. ex Griseb.	-
	R. revoluta Urb.	+++
14. Fam.:	Combretaceae	
	Bucida ophiticola Bisse	-
15. Fam.:	Convolvulaceae	
	Ipomoea argentifolia A. Rich.	-
16. Fam.:	Cyrillaceae	
	Cyrilla cubensis P. Wils. ex Britt.	-
17. Fam.:	Elaeocarpaceae	
	Sloanea curatellifolia Griseb.	-
18. Fam.:	Ericaceae	
	Lyonia latifolia (A. Rich). Griseb.	-
	L. longipes Urb.	-
	Vaccinium ramonii Griseb.	-
19. Fam.:	Erythroxylaceae	
	Erythroxylum alaternifolium A. Rich.	-
	E. minutifolium Griseb.	-

Table 1 (cont.)

20. Fam.:	Euphorbiaceae	
	Acidocroton acunae Borhidi	-
	Bonana suborbicularis Borhidi et Urbino	++
	Chamaesyce dorsiventralis (Urb.) Millsp.	-
	Croton bispinosus Wr. in Sauv.	-
	Ditta myricoides Griseb.	-
	Drypetes lateriflora (Sw.) Kr. et Urb.	-
	Euphorbia munizii Borhidi	-
	E. podocarpifolia Urb.	-
	Gymnanthes recurva Urb.	++
	Leucocroton comosus Urb.	++++
	L. ekmanii Urb.	++++
	L. havanensis Borhidi	++++
	L. moaensis Borhidi et Muñiz	++++
	L. obovatus Urb.	+++
	L. revolutus Wr. in Sauv.	++++
	Pera ekmanii Urb.	-
	Phyllanthus chamaecristoides Urb. ssp. baracoensis (Urb.) Webster	++++
	Ph. comosus Urb.	++++
	Ph. mirificus Webster	++++
	Ph. myrtilloides Griseb. ssp. shaferi (Urb.) Webster	++++
	Ph. pachystylus Urb.	-
	Ph. x pallidus (Wr. ex Griseb.) Webster	++++
	P. subcarnosus Wr. ex Muell.-Arg.	+++
	Savia clusiifolia Griseb.	+
	S. cuneifolia Urb.	+++
21. Fam.:	Fabaceae	
	Harpalyce acunae Borhidi et Muñiz	-
	H. borhidii Muñiz	-
	Sauvallella immarginata (Griseb.) Urb.	-
22. Fam.:	Flacourtiaceae	
	Caesaria crassinervis Urb.	-
	Xylosma acunae Borhidi et Muñiz	-
	X. buxifolium A. Gray ex Griseb.	-
23. Fam.:	Gentianaceae	
	Macrocarpaea pinetorum Alain	-
24. Fam.:	Gesneriaceae	
	Bellonia spinosa Sw.	-
	Gerneria norlindii Urb.	-
25. Fam.:	Goetzeaceae	
	Henoonia myrtifolia Griseb.	-
26. Fam.:	Goodeniaceae	
	Scaevola wrightii (Griseb.) Maza	-
27. Fam.:	Hypericaceae	
	Hypericum fasciculatum Lam.	-
28. Fam.:	Lauraceae	
	Guatteria cubensis Bisse	-

Table 1 (cont.)

29. Fam.:	Lobeliaceae	
	Siphocampylus patens Griseb.	-
	S. subglaber Urb.	-
30. Fam.:	Magnoliaceae	
	Talauma minor Urb. ssp. minor	-
31. Fam.:	Malphighiaceae	
	Byrsonima biflora Griseb.	-
	B. coriacea (Sw.) DC.	-
	B. minutifolia Alain	+
	B. orientensis Bisse	-
	Stigmaphyllon nipense Howard	-
32. Fam.:	Melastomataceae	
	Calycogonium grisebachii Triana	-
	C. rosmarinifolium Griseb.	-
	Mouriri emarginata Griseb.	-
	M. valenzuelana A. Rich.	-
	Tetrazygia coriacea Urb.	-
33. Fam.:	Menispermaceae	
	Hyperbaena longiuscula Mold.	-
34. Fam.:	Myricaceae	
	Myrica shaferi Urb. et Britt.	-
35. Fam.:	Myrsinaceae	
	Rapanea ferruginea (Ruiz et Pav.) Mez	-
36. Fam.:	Myrtaceae	
	Calyptranthes capitulata Wr. in Sauv.	-
	C. monocarpa Urb.	-
	Eugenia cycloidea Urb.	-
	E. piedraensis Urb.	-
	E. rigidifolia A. Rich.	-
	Myrcia susannae Borhidi	-
	M. valenzuelana (A. Rich.) Griseb.	-
	Myrtus ekmanii Urb.	-
37. Fam.:	Nyctaginaceae	
	Guapira ophiticola Borhidi	-
	Pisonia byrsonimifolia Heimerl et Ekm.	-
	P. cajalbanica A. Diaz	-
	P. petiolaris Heimerl et Ekm.	++
38. Fam.:	Ochnaceae	
	Ouratea revoluta Wr. ex Griseb.	-
39. Fam.:	Oleaceae	
	Chionanthus cubensis (P. Wils.) Stearn	-
	Ch. domingensis Lam.	+
	Ch. moncadae (Borhidi et Muniz) Borhidi	-
40. Fam.:	Piperaceae	
	Piper holguinianum Trel.	-
41. Fam.:	Poaceae	
	Aristopsis bissei Catasus	-

Table 1 (cont.)

42. Fam.:	Polygalaceae	
	Polygala guantanamana Blake	-
	P. oblongata (Britt.) Blake	-
43. Fam.:	Polygonaceae	
	Coccoloba cowellii Britt. et Wils.	-
44. Fam.:	Rhamnaceae	
	Karwinskia oblongifolia (Britt. et Wils.) Urb.	-
	K. orbiculata (Britt. et Wils.) Urb.	-
	Reynosia retusa Griseb.	-
45. Fam.:	Rubiaceae	
	Acrosynanthus latifolius Standl.	-
	Antirhea multinervis Urb.	-
	A. scrobiculata Urb.	-
	Ariadne shaferi ssp. shaferi (Standl.) Urb.	+++
	Casasia nigrescens (Griseb.) Wr. ex Urb. ssp. nigrescens	-
	Chiococca cubensis Urb.	-
	Ch. parvifolia Wullschl. ex Griseb.	-
	Coussarea urbaniana Standl.	-
	Exostema obovatum Griseb.	-
	Guettarda clarensis Britt.	-
	G. valenzuelana A. Rich.	-
	Neomazaea phialanthoides (Griseb.) Kr. et Urb.	-
	Phyllomelia coronata Griseb.	+++
	Psychotria lopezii Acuña et Roig	+
	P. shaferi Urb.	-
	Randia cubana Borhidi	-
	Rondeletia camarioca Wr. in Sauv.	-
	R. pachyphylla Kr. et Urb.	-
	R. sp.	++++
	Schmidtottia cubensis (Standl.) Urb.	-
	Scolosanthus lucidus Britt.	-
	Shaferocharis multiflora Borhidi et Muñiz	-
	Suberanthus brachycarpus (Griseb.) Borhidi et Fernandez	-
	S. canellifolius (Britt.) Borhidi et Fernandez	-
46. Fam.:	Rutaceae	
	Zanthoxylum nannophyllum (Urb.) Alain	-
	Z. phyllopterum (Griseb.) Wr. in Sauv.	-
47. Fam.:	Sapindaceae	
	Allophylus cristalensis Lippold	++
	Thoninia punctata Radlk.	-
48. Fam.:	Sapotaceae	
	Chrysophyllum clarense Urb.	
	Dipholis cubensis (Griseb.) Pierre	-
	D. jubilla Ekm. ex Urb.	-
49. Fam.:	Simaroubaceae	
	Alvaradoa arborescens Griseb.	-
	Simarouba laevis A. Rich.	-
50. Fam.:	Solanaceae	
	Brunfelsia linearis Urb.	

Table 1 (cont.)

51. Fam.:	Symplocaceae Symblocos strigillosa Kr. et Urb.	-
52. Fam.:	Theaceae Ternstroemia flavescens Griseb.	-
53. Fam.:	Theophrastaceae Jacquinia robusta Urb.	-
54. Fam.:	Thymeleaceae Daphnopsis oblongifolia Britt. et Wils.	-
55. Fam.:	Ulmaceae Trema lamarckiana (R. et S.) Blume	-
56. Fam.:	Verbenaceae Pseudocarpidium ilicifolium (A. Rich.) Millsp.	-
57. Fam.:	Violaceae Hybanthus havanensis Jacq. ssp. serpentini Borhidi	+

Conclusions

1. The results confirm the conclusions postulated in the first preliminary article based on the study of 164 belonging to 23 falmiles.

2. The main hyperaccumulator familes are: Euphorbiaceae, Buxaceae, Asteraceae and Rubiaceae.

3. The most important hyperaccumulator genera are: Leucocroton (Euphorbiaceae), Phyllanthus (Euphorbiaceae), Buxus (Buxaceae), Senecio sl. (Asteraceae),

4. Among the 24 serpentine endemic genera of the Flora of Cuba, only two monotypic Rubiaceae are hyperaccumulators: Ariadne and Phyllomelia.

5. New hyperaccumulator families are: Clusiaceae (Rheedia), Oleaceae (Chionanthus).

6. New hyperaccumulator genera: Bonania (Euphorbiaceae), Savia (Euphorbiaceae), Eupatorium s.l. (Asteraceae), Rondeletia (Rubiaceae).

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The authors are indebted to Prof. Roger REEVES (Massey University, New Zealand) for the informations about the results of the parallelly carried quantitative analysis of the same materials. The study was executed within the framework of the Cuban-Hungarian Acedemic agreement and supported by the Hungarian Scientific Research Fund (OTKA No. 1299).

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BOOK REVIEWS

Editor: K. T. KISS

ALLEN, M. F.: The Ecology of Mycorrhizae. Cambridge University Press, Cambridge, New York, Port Chester, Melbourne and Sydney 1991, 184 pp.

As important partners of plants mycorrhizal fungi play role in every terrestrial ecosystems. Some of the articles and discussions have been published since late 1800s omitted the incorporation of mycorrhizal interactions into the general ecological literature. Many ecologists have ignored the interpretation of mycorrhizal relationships in their own research. ALLEN's book is an interesting attempt to relate the biology of mycorrhizae to many aspects of ecological considerations and to show the importance of mycorrhizal associations to many of specialists dealing with either theoretical or practical aspects of ecological problems. The author presents in 8 chapters the ecological characteristics of mycorrhiza. The first one gives detailed information about the types of symbioses between organisms with special regard to one of these interaction, the mutual symbioses between plant and fungus namely mycorrhiza. In the second chapter the author describes the types of mycorrhizae and their distribution, the structure of both symbiotic partners and discusses their structure-functioning relationships in the environment. Supporting by paleobiological data the third chapter points out the hypothetical role and importance of mycorrhiza in the evolution of plants and their survival and expansion in different habitats. In this part the author describes how did the micorrhizae evolve concerning their molecular biology. The next chapter describes the affects of symbioses on the physiology growth and reproduction of each of the mutualists, plant and fungus. As important factors on survival discusses the author the dispersal and establishment of mycorrhizal fungi into new habitats and the establishing new mycorrhizal associations. Many plant community types are well recognized but to describe a single "microbial community" is actually impossible. Therefore the author takes the approach of community processes in the context of mycorrhizal associations rather than delineating a "mycorrhizal community". Using this approach the author reviews in the fifth chapter the literature that deals with responses of the community to mycorrhizae including plant-fungal and animal-fungal interactions. The sixth chapter deals with the role of mycorrhiza played in the dynamics of ecosystems. In addition this chapter gives information about the distribution of mycorrhizae in different biomes and their relationship to carbon dynamics, nitrogen and phosphorus cycling of ecosystems. At the end of this chapter discusses the author the effects of mycorrhiza on vegetation units. In the seventh chapter the author summarises the significance of mycorrhizal symbiosis in the successional processes, but there remain several questions have to be answered concerning how alter the micorrhizae the rates and direction of succession. Finally, the last chapter gives outlines for perspectives and future directions in the mycorrhizal research.

This book illustrated with 51 figures and photos, completed with a huge number (556) of references is a thorough, up-to-date work, remarkable and useful reading matter. In can be recommended not only to ecologists, mycologists working in the fields of mycorrhiza research but to foresters, agriculturists dealing with practical aspects of mycorrhiza.

CS. LOCSMÁNDI

BAILEY, J. A.—JEGER, M. J. (eds): Colletotrichum, Biology, Pathology and Control. C.A.B. International, Wallingford 1992, 388 pp.

Colletotrichum is one of the most important and widespread genera of plant pathogenic fungi. It could cause diseases on many crops, but these fungi have also been used in studies of pathogene-host interactions and some of them are being developed as mycoherbicides, too. This volume summarizes the most important information about this important fungal genera.

A taxonomic overview is given at first dealing with the current status of the described hundreds of "species" and "genera" both for the Colletotrichum anamorphs and the Glomerella teleomorphs. Later one could find a paper about sexuality and genetics of Colletotrichum species and another about genome analysis of this fungus including the possibilities of molecular transformation. The next three chapters contains informations about pathogenesis as role of lectins, the infection strategies and the mechanisms of resistance to these fungi. The next part of the book (Chapters 7—15) deals with the diseases caused on different hosts by different Colletotrichum species. Among of most important species some are important also in Hungary, e.g. C. coccodes, C. graminicola, L. lindemuthianum, while some diseases are rather important in the tropical areas. Molecular analysis of C. gloesporioides isolates from different hosts are also given. Epidemiology of this species in the Tropics is presented in Chapter 16. Chapters 17 and 18 are referring to control medhods of Colletotrichum diseases. We could read about chemical control of infection in mangoes at first, while strategies and prospects for biological control are mentioned in the next. On the other hand Colletotrichum might be accepted for biological control of weeds. This is reviewed in last part of the book (Chapter 19). Some species or forms have a highly specific pathogenic potential on some weed species and these isolates are probably used for mycoherbicides.

The volume, by its complexity, would be of interest a wide range of readers working areas of mycology, soil biology, plant ecology and phytophathology.

E. I. SIMAY

BARBOUR: Terrestrial Vegetation of North America. Cambridge University Press (publication date: 12. 04. 1990) (0 521 38678 0). Price: Ŀ 20

Writing concisely about a broad topic that should deserve many more pages because of its complexity, but yet has to be squeezed into the limits of only one volume, is not an easy task. Especially not, when the topic is the vegetation of a whole continent with its enormous varietg in climate, topography, geology and, as a consequence, vegetation. The editors of the Terrestrial Vegetation of North America made an impressive job reducing the book's content to a treatable size, but still maintaining the high quality of the individual chapters. Each of the thirteen chapters describes one main vegetation formation of North America from the Arctic tundra to the warm deserts. Specific attention has been paid to such less known formations as the Americal chaparral, the inter-mountain deserts, the evergreen forests of the Pacific Northwest and the south-eastern coastal vegetation. The general structure of the chapters is surprisingly uniform despite the fact that numerous authors have contributed to this volume. The title page of the chapters presents a distribution map (the print sometimes is very light) of the discussed vegetation formation. Then the authors briefly overview the climate, soil types, geology and — if relevant — the glacial history or topography of the discussed region with specific emphasis on the variation in these factors. The main part of the chapters contains a quite detailed description of the vegetation types within the formation including lists of dominant species analysis of vegetation cover and vegetation structure. Although the terminology used does not follow the traditions of the European phytosociologist school, the vegetation types may be easily parallelled with their European equivalents. Overall, these sections provide a fairly complete picture about composition, structure and general physiognomy of the various vegetation types. But this is still not all about, since the second half of the chapters address several topics in different ecological processes, such as succession and primary production, fire ecology, life history characteristics and ecophysiological adaptations of dominant species. Each chapter is self-contained presenting the most recent information and results of ecological research on the discussed vegetation types. The book is very well illustrated with numerous graphs, maps, tables and sometimes excellent black and white photographs of vegetation or typical species, which enhances even more the utility of the book. However, I also have to mention some weaknesses of this volume. First, the distribution maps on the title pages are not precise enough relative to one another; unfortunately, there are areas, such as South Texas that are not covered at all with any of the discussed vegetation types. On page 256, for instance, the grasslands of Texas extensively overlap with the

Chihuahuan desert in the Rio Grande—Pecos region. I also missed the very informative climatic diagrams from the tundra, taiga and deciduous forest chapters. I was also not too satisfied with the chapter about American grasslands. My curiosity, and perhaps others' as well, was not fulfilled by the relatively short treatment of this vegetation. I think that the prairie, as one of the most famous and best-investigated vegetation types of North America, should have deserved a much more thorough analysis (the role of fire, for example, is discussed in three short paragraphs altogether), better photographs and also some additional information on environmental factors including climatic diagrams or data tables, which would be essential to the understanding of the development and maintenance of this vegetation. And finally one puzzling question: Why is a whole chapter devoted to the Tropical and Subtropical vegetation of Meso-America? I do not see any reasons, for which Central America should be discussed here except for the fact that there is certainly no sharp boundary in the vegetation between North and Central America. Yet, I think Central America Should rather be discussed in a different volume with the vegetation of South America. Although the books title suggests a biogeographic-phytosociological approach, the entire volume is somewhat biased toward the ecological aspects of vegetation science. The book successfully combines the conventional vegetation descriptions with the most significant results of the latest directions in ecological research including vegetation dynamics, succession, ecophysiology and evolutionary ecology. The editors recommended their book to professional ecologists, as well as to advanced undergraduate and graduate students. Indeed, this book is perhaps the most up-to-date compilation of the North American vegetation with strong emphasis on the underlying ecological processes. These strengths make the volume and invaluable reference for those who are deeply interested in the topic, and also an excellent supplementary source for courses on the North American vegetation.

G. LENDVAI

BELL, A. D.: Plant Form: An Illustrated Guide to Flowering Plant Morphology. Oxford University Press, Oxford—New York—Tokoyo 1991, 341 pp.

This book is an attractive, richly illustrated dictionary of flowering plant morphology. From one side, it can be regarded as a basic reference of this well-established discipline. But, from the other side, it is also a treasury of new concepts in studying plant form. Instead of the 'static' view of traditional morphology, where the main emphasis is laid on the description of existing structures, A. D. BELL concentrates on the process of forming these structures, by focussing on the basic principles of their construction. In contrast with the classical 'sterile' view of plant form, he aims to examine development of the different structures in interaction with their environment. Many of the topics are of evolutionary or ecological relevance. The book is divided into two sections. The first, Morphological description, is a detailed account of the morphology of leaves, stems, roots, and reproductive organs, together with their possible modifications. He details several special functions performed by those organs, including not only peculiar — often surprising — parts of plants, but also widespread, and very important means of clonal multiplication. Additional parts are devoted to some taxa of 'difficult' morphology, like grasses (including bamboos), sedges, orchids and cacti. The book does not lack a certain kind of self-irony and sincerity, thus, several pages deal with 'misfits', i.e. those 'abnormal' plant structures that can not be accomodated in the rigid frame of morphological terms.

The second section, Constructional organisation, is a fascinating introduction into the dynamic aspects of morphology. On the basis of position and activity of meristems, it explains how developmental rules are translated into the characteristic structures of plant body. Modular organisation of plants provides a common basis for a comprehensive treatment of growth patterns, and the resulting architectural types for trees, lianes and herbs. Some exciting topics, like plant behaviour or the applicability of the Fibonacci series for describing phyllotactic patterns might claim special interest.

As a consequence of the author's view, a common fault of morphological textbooks is avoided here: the exaggerated emphasis on floral structures. Although a bias towards reproductive organs can be understood from the viewpoint of their taxonomic importance, now it is a

good experience to read this well-balanced, comprehensive work. The author criticizes another bias in the view of the average European morphologist: a preoccupation with European species. Therefore, he intends to show a wide range of plant forms from all continents, especially from the tropics. This makes the illustrations highly informative and fascinating.

This book is a well-structured, easy to use guide, with many cross-references. The topics are arranged in a dictionary form: every title occupies exactly one double-paged spread, as a result of the excellent editing. the odd pages are wholly devoted to illustrations: drawings by Alan BRYAN, and photographs by the author. These photos are to be appreciated not only for their scientific but also for artistic value.

I think, most of the readers would take this book in hand not only for gaining scientific information, but for enjoying the attractive world of plant forms presented here. According to the intention of the author, it can be recommended to both experienced scientists and to curious amateur plantsmen. It can also be used with profit as a textbook for university courses.

B. OBORNY

BELL, P. R.: Green Plants. Their Origin and Diversity. Cambridge University Press, Cambridge 1992, 315 pp.

The book is a revised edition of the earlier Diversity of Green Plants by the author and C. L. F. WOODCOCK.

It is a trend in textbook writing on the area of plant biology dealing with plant diversity to present the plant kingdom as a whole. It is possible only with the presenting of evolutional relationships among the taxa. Furthermore this consideration cannot allow to eliminate the fossils. In this respect the book is a good textbook of anatomy, morphology, palaeobotany and plant evolution at the same time.

The text after a brief characterization of the plant kingdom ranges the plants from the simplest photosynthetic organisms to the highly complex flowering plants following the way of evolution throughout more than 3 milliard years.

Algae are grouped into three subkingdoms according to their photosynthetic pigments. Each chapter presents the most important characteristics of the division considered at the beginning, such as habitat, pigments, food reserves, cell wall components, reproduction, growth forms and specials.

Among the algae containing chlorophylls a and b Prochlorophyta and Chlorachyanophyta divisions can also be found, which are among the newest discoveries of the latest time giving further help to the understanding of evolutional connections considering the photosynthetic pigment molecules.

The book does not discuss the fungi being not green, and only mentions the lichenes, but devotes to Bryophyta subkingdom 25 pages.

Two-third of the book deals with Tracheophyta subkingdom dividing them into four groups considering their reproductive organs. The parts do not only display the groups but summarize the general characterization of the Tracheophytes and present more or less data relating to their evolutional aspects. Flowering plants got only less than 20 pages without any classification. The diversity of this plant group dominating in the recent flora deserves an another book.

The book contains the latest advances in the topic concentrating on the phylogenetic relationships with the tools of cell biology, anatomy and palaeobotany. The text is supported by well-done tables, drawings and photos. At the end of the book an excellent glossary, book suggestions and an index help the reader.

The book is recommended for students at higher education and all colleagues interested in botany.

M. PAPP

BRÜCHER, H.: Useful Plants of Neotropical Origin and Their Wild Relatives. Springer, Berlin 1989, 296 pp., 252 figures and photos (b—w)

If we compare Eurasia with the American continent, we notice a fundamental difference as far as the origin of our cultivated plants and domesticated animals is concerned. Whilst almost all useful animals have been domesticated in the 'Old World', a considerable amounts of essential food plants and industrial crops originated from the 'New World'.

The American continent served as a vast treasury of useful plants, while the Amerindian tribes of Meso- and South America were talented by a notable breeding ability in forming these potentials into precious renewable source of cultivated crops.

Columbus and his crew were deeply amazed by the diversity of exotic fruits, vegetables and spices which the indians presented to them on their first voyage. Although he never find the short seaway to the pepper islands of Asia, Columbus was lucky to receive from these tribes an other pepper plant (Capsicum sp.) which shortly after this became known as the 'Spanish pepper'. Besides this, a multitude of other useful plants arrived from America and spread to Europe, Africa and Asia.

This valuable book deals with most of them: 176 species or group of species. The material is arranged into practical groups:

- roots and tubers, providing carbohydrates,
- farinaceous plants,
- protein plants,
- oil plants,
- palms,
- industrially used plants,
- aromatics, narcotics, stimulants, spices,
- timber species,
- forage grasses and legumes,
- fruits.

One can find in each section well-known groups like potato, maize, beans or tomato as well as lesser known ones like amaranth, quinoa, arrakacha, tawri or jicama. Besides these this book will provide surprises even for experts, several dozen species-groups virtually unknown as useful crops, like Calathea, Maranta, Ullucus or Bromus species.

But even in known groups of potato or paprika the reader may find many 'new' species or land races. The importance of these valuable genetical sources cannot be overstated: these are the most precious heritage of the mankind. Comparing to their importance they are surprisingly 'local' crops, known and used in a small geographical area only. It is the great merit of the author to share his rich experiences gathered during decades of living and exploring in tropical latitudes in the neotropics. One can only compare the accumulated scientific, practical and even ethnobotanical information of this book to a forgotten and rediscovered ancient treasury: it is full of unpolished, dust-covered gems and precious stones.

Although the plants covered are of tropical origin, their area of cultivation in certain cases has been extended far beyond the true topics, deep into the temperate zone, even to its northern edge (e.g. potato). In other cases one may only guess the same capacity, but nobody has ever tried to explore their limit of introduction. It can be said for sure, it would be the best investment to try to indroduce many of them even in the countries of the temperate zone, not to speak about the tropical countries.

The text is well written, informative, concise; the compilation is practical, and the quality is high, as usual at Springer. Each section is followed by references.

The usefulness of the book could have been increased by adding a list of research contacts for each plants as N. VIETMEYER did in his similar books. In this case the interested reader may wonder how could be obtain seeds or tubers from the species he may wsh to try to introduce in his own country.

I can recommend this great book to specialists, experts, scientists, agronomists, but also to interested people of the American continent to be proud of their rich heritage and treasures to be distributed for all the mankind's benefit in the future.

Z. SZŐCS

CASAS, C.—BRUGUES, M.—CROS, R. M.—SERGIO, C.: Cartografía de Briofits. Fasc. I.: 1—50. Institut d'Estudis Catalans, Barcelona 1985, 154 pp.

The number of biogeographical and taxonomical works and studies on the Spanish—Iberian Peninsula is incrasing in the last few years. Bryology I—II the study of bryophytes has also started to develop fast, and many Spanish and Catalan biologist specialized in this field. Many foreign scientists who visited Spain also provided a lot of new data. After the publication of a Moss Check list, edited by CASAS in 1981, a complete, current — up-to-date catalogue was the next important thing to be prepared. The authors of this book believe that their cartographic work can fulfill this requirement. Furthermore a clearer, more specific picture is given regarding the geographical localization and the range of occupied territories of each species, with the help of the species distribution maps. This work was also inspired by the fact, that the Work group of mapping the Bryophytes of Europe asked the Barcelona bryologist team to map the peninsula's bryophytes. The Spanish Peninsula and the surrounding islands represent a geographical unit at the Southernmost and Westernmost part of Europe. This and the closeness of the African shores influence the amount and quality of the bryophytes, therefore it is quite varied. It comprises of Atlantic, Mediterranean, Irano-Turanic, Central-European and Arcto-Alpine elements with many indigenous and relic species.

The book was written in English and Catalan. The first volume describes 50 species. The species are gathered in alphabetical order, and certain species are picked to show examples for the different types of distribution areas. The book lists indigenous species, ones with disjunct areas, circumboreal, Mediterranean, Atlantic, alpine species. The 50 exquisite maps divided by 10 x 10 km UTM screens, use different marks for points taken before and after 1950, from herbarium or only bibliographic data. There is a short ecological and bryocenological characterization for each species, which is followed by a complete citation list. We can also find a schematic contour map in the book. If we place this map over the others we can get information about the heights distribution of the species. The book also includes a map showing the county borders, and another showing the positions of the islands.

Overall this book is the first result in the processing of a great amount of work. It is very practival and thorough, and therefore exemplary for any other work in botanical cartography. The authors would like to continue their work; increase and improve the catalogue taking the new biogeographical studies into consideration, and adding the species newly found on the Spanish Peninsula.

I recommend this book for every botanist who works on plant distribution or cartography.

B. PAPP

COURTRIGHT, G.: Trees and Shurbs for Temperate Climates. Timber Press, 3rd Ed. Inc. Portland, Oregon 1989, 239 pp., 770 colour photographs

COURTRIGHT has been a licensed nurseryman in California for 38 years. In 1976 he sold his business and he completed this book, a dream of 25 years. The author has also been the show designer and superintendent for 34 flower shows in California in the last 15 years.

When COURTRIGHT published his first booklet on this subject be designed it to meet his nursery customers' need. He understood their needs, based on questions asked while shopped for plants. It was published to help the home gardener choose the correct plant for any given place in the garden. That booklet was quite well used through many years, but because few people can visualize plants from verbal descriptions, the author determined to his next book well illustrated. The result of much work and travel to take pictures is the Trees and Shrubs for Temperate Climates.

Unlike other gardening books that usually are organized by plant families this one have divided the plants into sections by typical height, and type, i.e. Low-Growing Shrubs, Medium-Growing Shrubs, Tall-Growing Shrubs, Trees, Vines and Conifers. Each plant is accompanied by a colour picture. Although this photograph can be very shady and even a well-trained botanist would not be able to recognise the different species it helps the reader imagine the shape of

the actual plant. These photographs were designed for the gardeners, not for the botanist. Nevertheless those really useful for landscape designers as they give an impression of what might be expected the plant to be look like in the garden.

There are two numeric planting indexes for each plant. The first is a temperate guide, shown as temperate zone below which a plant cannot withstand the cold. The second is a planting guide number which indicates the broad cultural requirements of the plant — exposure to sun, drainage and soil requirements. The planting guide classifies the plants by these requirements into five groups. These planting instructions are general since individual conditions vary. The temperature rattings are for the zone 3-10 (from -40 F till 40 F). Here again, of corse these temperatures suggested in this book are approximate. The book also contains a guide to botanic name with pronunciation dictionary and one of the same kind for common name to botanic name.

The chapters are put together according to the shape and the colour of the flower of the plant. There is a chapter which contains the low-growing shrubs like Azaleas. Although these are referred to by botanists as Rhododendrons, they are called Azaleas in this book because it is the way they are described in the wholesale nursery catalogues. Chapter by chapter it uses the alphabetical order to describe a plant genus or that particular one which belongs to that special measurement. There is a smaller chapter dedicated to Ferns. included in the Medium-Growing shrubs. Unfortunately Asparagus genus is listed within the other ferns although there is a short indication that these are not true ferns. Because of the confusing alphabetical order the Forsythia belongs to the Fuchsias without any indication that they are not in the same family. There are plenty of these inaccuracies which make the book less useful for a botanist than a nurseryman.

Since the author of this book claims that it is intended to be a visual plant dictionary therefore it is really useful for gardeners. It must be one of the most extensive collections of coloured photographs and descriptions of landscape plants ever published, and it can useful for botanists and in university education as well.

R. GOLDMAN

CROS, R. M.: Flora briologica del Montnegre. Institut d'Estudis Catalans. Barcelona 1985, 287 pp.

The Catalan Institute of Sciences, which published this book, awarded it the prize named after the great Catalan botanist Pius Font i Quer, in 1982. The book explores the bryophyte flora of Mountain Montnegre. Although the book was written in Catalan, it is understandable and easy to follow for those scientists, who are familiar with either Spanish or French.

Montnegre is situated North-East of Barcelona, between the rivers Tordera and Areys de Mar, at the Catalan sea shore. It is a low mountain, the highest point being 757 m. Its base rock is granite and it lies in an area of mediterranean climate. The vegetation is quite homogeneous with a few eurosiberian elements mixed in the mainly mediterranean flora composition. The main part of the hill is covered with macchia vegetation, the mesophyl forests appear only above 600 m. Beeches are formed around the highest peaks. There are several streaks intersecting and surrounding the mountain, therefore riverine vegetation also covers a large area.

This area was chosen for the study of bryophytes, because of its closeness to Barcelona, hence the possibility to do fieldwork several times a year. Also, as it is not easily accessible, few botanists visited the place before, and a thorough sampling was hoped to reveal many interesting data regarding the moss flora. The flora catalogue in which the ecological relations of the different habitats are considered, and the ecological demands of the species are also recorded, is the result of a year's systematic investigation. It contains 213 species (of which 151 belong to Musci, and 63 are from Hepaticae), and their precise locations are given by the UTM screens. At the end of the book a comparison of the moss flora of Montnegre with that of other close mountains (Montseny, Collserola) is given.

This book gives a good review of the bryophyte flora of different habitats on this low mediterranean mountain. It provides useful information for botanists and bryologists, who deal with the vegetation of submediterranean and mediterranean areas.

B. PAPP

DE KROON, H.—VAN GROENENDAEL, J. (eds): Clonal Growth in Plants: Regulation and Function. SPB Academic Publishing, The Hague 1990, 196 pp.

Clonal development plays an important role in the ontogeny and phylogeny of various taxa in vascular plants. Its occurrence is not exceptional, indeed, clonality seems to derive from the basic principles of the organisation of plant body. It is widespread in nature: as estimated, in an average non-forested community in the temperate zone about 70% of the species are clonal. Thus, clonal growth rules may largely influence the spatial pattern and stability of many plant communities. On the side of ecology, this is one of the reasons for the increasing need for understanding the mechanisms that govern clonal growth, and the growing effort to utilize information from concerned disciplines, like physiology or evolutionary biology.

This book presents ten papers from the material of the international workshop held at Schin-op-Geul, The Netherlands, in 1988. It integrates many areas, ranging over plant morphology, physiology, ecology and evolutionary biology. The chapters are written by leading experts on the subjects, who introduce the reader into some recently emerging questions related to clonality on their own fields. A common feature of the various viewpoints that clonal characteristics are examined in relationship with the environment, and thus — as exposed in the title — they aim to account for the causal background of forming different clonal structures.

The chapters are grouped into two sections: (1) Regulation and (2) Function. As introduced by the editors, the first part, Regulation, aims to collect information on the ontogeny, phylogeny, demography, resource transport, meristem differentiation, and hormonal control of clonally growing plants. The second, Function, concentrates on resource partitioning, placticity, and natural selection. However, the distinction between these two aspects is not obvious, especially when — as in the case of many papers — the evolutionary background of a clonal characteristic is discussed, where the inner constraints of development meet environmental effects. Thus, the reader may feel that organisation of the chapters in unclear.

This book is a highly informative reference. One of its greatest merits is that various plant phyla are involved in the chapters. H. J. DURING gives a comprehensive account of clonal growth forms, and special means of vegetative spreading in bryophytes. B. A. CARLSSON et al. present experiments on a stoloniferous and rhizomatous pteridophyte (contrasted with an angiosperm), in a study on the adaptation to arctic environments. M. MOGIE and M. J. HUTCHINGS present an overview on the distribution of clonality on the phyletic tree, and the different ontogenetic constraints that determine the incidence of clonal growth in many groups. They also discuss the taxonomic distribution of the different structures by which clonality is achieved, and relate them to the organisation of meristematic activity.

The book highlights many physiological and morphological aspects of the genet/ramet relationship. C. MARSHALL reviews experimental results on the pattern of transport between interconnected ramets, and the consequences of physiological integration in the genet, with regard to optimal exploitation of environmental resources. H. DE KROON and F. SCHIEVING describe three basic types of resource use, where the aspects of resource partitioning are related to cloonal growth strategies. These growth strategies — as described by developmental rules —, that govern the spatial arrangement of ramets, are reviewed in the paper of W. J. SUTHERLAND and R. A. STILLMAN. Comparing models with experimental evidences, M. J. HUTCHINGS and M. MOGIE discuss the control and consequences of spatial structures.

Considering the above detailed hierarchical construction of clonal plants, these organisms show several characteristics that cannot be interpreted in the traditional view of life history theory. M. A. WATSON investigates the relationship between the timing of critical developmental events and the demographic response to environmetal change, re-examining the life history of a clonal species. O. ERIKSSON and L. JERLING outline some special features of clonal organisms, introducing a new concept for fitness, and examining the hierarchical nature of selection and the possibility of 'risk spreading' in the genet.

The papers are supplemented by an excellent evaluation by the editors. Although the book cannot be considered as a systematic discussion of clonal growth, especially because most of its topics are undergoing rapid development in recent times, it is certainly a good collection

of reviews, that provides an interesting cross-section of the issues in focus at present. It can be recommended to both researchers and students working in any of the fields concerned.

B. OBORNY

DILLARD, G. E.: Freshwater Algae of the Southeastern United States. Part 3. Chlorophyceae: Zygnematales: Zygnemataceae, Mesotaeniaceae and Desmidiaceae (Section 1). Bibliotheca Phycologica 85. J. Crammer in der Gebrüder Borntraeger Verlagsbuchhandlung, Berlin, Stuttgart 1990, 172 pp.

This book is the third part of a series which is planned to present a compendium of the freshwater algae (excluding the Bacillariophyceae and Charophyceae) of the Southeastern United States.

At the fore-part of the book there are key to classes, key to the orders of the Chlorophyceae, and a short description of order Zygnematales. The book reports on 20 genera (Spirogyra, Sirogonium, Debarya, Mougeotia, Zygogonium, Zygnemopsis, Zygnema, Mesotaenium, Spirotaenia, Cylindrocystis, Netrium, Roya, Genicularia, Gonatozygon, Penium, Closterium, Docidium, Pleurotaenium, Triploceras and Tetmemorus). More then 400 taxa are described in this volume with figures (altogether 51 plates), synonyms in all cases where warranted corrections were known, distributional data for each subgeneric category, and literature citations for each genus based on 272 references. "Accurate illustrations of algal taxa, based on authoritative sources, are of critical importance to the usefulness of a work such as this" as we can read in the introduction of the book.

This book is very useful to identify some filamentous green algae, which are found very often e.g. periphytic samples, but this book is a bit difficult to use. In one respect it can be missed the habitat of species, on the other hand most of the described species can be found very rarely in reproductive stage. Many keys of species are ramified on the bases of e.g. zygospores, gametangium, conjugation, etc. Beside this there are many morphological keys too, so I can recommend to every algologist and hydrobiologist who deals with algae identification.

É. ÁCS

EDWARDS, A. R. (ed.): The Oamaru Diatomite. Contributors: A. R. EDWARDS (NZGS, Lower Hutt), A. J. DOING (Dunedin), N. DE B. BORNIBROOK (Nzgs, Lower Hutt), P. A. MAXWELL (NZGS, Lower Hutt) and F. S. C. REED (Christchurch). New Zealand Geological Survey Paleontological Bulletin 64. Lower Hutt, New Zaland 1991, 260 pp.

This book contains the summary of investigations on the worldwide known Upper Eocene diatom-bearing sediments of Oamaru, with special emphasis on its age, stratigraphic position and paleoenvironment.

Part 1. Stratigraphy and Paleoenvironment. A. R. EDWARD (in Chapters 1 and 3—6), and N. DE B. HORNIBROOK (in Chapter 2) describes the geological formations of the Alma Group, their correlation and stratigraphic classification. Detailed descriptions of 20 Oamaru Diatomite sequences (location, stratigraphy and fossils) are given and their correlation discussed. The Oamaru Diatomite is Late Eocene (Runangan) and Early Oligocene (earliest Whangaroan) in age. Chapter 5 contains the data and interpretations on paleoenvironment and sedimentation. Chapter 6 (economic geology): possibilities of industrial usage and commercial prospects are outlined here. There are 49 figures, 12 plates and 2 maps (including the geological map prepared by the New Zealand Geological Survey).

Part 2. Paleontological contributions. Chapter 1. Philip A. MAXWELL: Nannofossils from Oamaru. Coelenterates, brachiopods, bivalves, cirripeds, crinoids and echinoderms are described, and illustrated by 2 tables, providing information on age and depositional depth. Chapter 2. A. J. DOIG: Diatoms from Oamaru. Quantitative evaluation of the diatom assemblage

of Oamaru. There are 3 main dominance zones: Stephanodiscus, Coscinodiscus and Melosira Zones. The method of extraction is described, the fossil assemblages are tabulated by localities, and peak zone correlation of the main Oamaru Diatomite sections are shown. Chapter 3. F. S. C. REED: Atlas of Diatomite. The best-known diatom forms are illustrated by 279 excellent microphotographs on 20 plates. Three new combinations are published, and references concerning the Oamaru Diatomite and its siliceous microfossils are listed. Chapter 4. A. R. EDWARDS: The Oamaru Diatomite (1876—1985). History of research with full bibliography. The checklist contains 1200 fossil taxa, belonging to 18 groups (Archaeomonadaceae, Brachiopoda, Bryozoa, calcareous mannoplankton, Cirripedia, Coelenterata, Diatomaceae, Ebriaceae, Echinodermata, Foraminifera, Mollusca, Ostracoda, Porifera, Radiolaria, Silicoflagellatae, trace fossils). The bibliographic references for taxa, and list of recent and ancient localities are included.

This volume was prepared in a faw-away country, on a closed research area, and the results were presented in a concise, easily understandable way by editors and contributors alike. The high-quality presswork of plates and tables make the book even more valuable.

M. HAJÓS

EL-BAZ, FAROUK (ed.): Deserts and Arid Lands. Martinus Nijhoff Publ., Dordrecht 1986, 222 pp.

This volume is the first one of the series: "Remote Sensing of Earth Resources and Environment".

Deserts are specially suitable objects for remote sensing studies because of their immense size and inaccessibility to detailed study by conventional methods.

This book contains 11 articles of different authors — 11 excellent examples of the utility of aerial photographs and space images in the study of arid and semi-arid terrains. Emphasis is placed on the physical features and terrain types using the examples from around the world and even the surface of the Mars.

The invited authors are renowned specialists of the remote sensing and the deserts.

To the general reader, this book is a review our knowledge of the dry parts of the Earth, their classification and varied features, their geological evolution space and time, and their development potentials.

To the specialist, like ecologists, botanists, geologists, it is a detailed account of the deserts and arid lands in North America, north Africa, Australia, China, India, Arabia.

From the articles one can see the general view of the global desertification-process. Particularly interesting is H. E. DREGNE's article on North American deserts and their vegetation. The author argues that desertification is caused mainly by man's impact: overgrazing by livestock, wood cutting, salinization, erosion. At the same time, this process can be turned around: ecological restoration is possible in many cases. Slightly desertified land can be restored to full productivity, but in some cases this restoration is not economically feasible. It may seem paradox, but the hyper-arid zones, like Death Valley and parts of Sonoran Desert are only slightly desertified while some arid lands are more seriously threatened.

One can find also interesting examplex of mapping vegetation cover and its changes due to desertification by remote sensing methods from Arabia, China and India, too.

This valuable book is highly recommended to experts interested in remote sensing and its applications, and also to ecologists, geologists enganged with arid lands and deserts.

Z. SZŐCS

EMES, M. J. (ed.): Compartmentation of Plant Metabolism in Non-photosynthetic Tissues. Cambridge University Press, New York, Port Chester, Melbourne, Sydney 1991, 204 pp.

The volume contains selected papers of the conference organized by the Society for Experimental Biology in Edinburgh in 1989.

An overview on techniques of labelling, differential centrifugation, density gradient separation, and enzyme localization by microscopy is given together with some aspects of organelle isolation.

Recent information on some aspects of fatty acid and lipid biosynthesis, possible roles of lipid exchange proteins, fine localization patterns of involved processes are considered. Structure, synthesis and degradation of oil bodies are discussed with particular interest on possible stabilizing and signalling role of oleosin in storage and breakdown of oil bodies. Role of storage proteins and glyoxysomes in the early stages of germination is outlined, their structure and biogenesis is described. Features of plastid and cytosolic forms of several isozymes, mechanisms affecting the ratio between these forms, and consequently, activities of different tissues are discussed. Interconversion of C-6 and C-3 augar phosphates, significance of the process in regulation of the cell's energy and carbon supply and in the sucrose-starch interconversion is described. Pathway and compartmentation of starch synthesis in developing grains is detailed with particular interest on the redistribution of C-1 and C-6 carbons. Flexibility of plant cell metabolism characterizing the phenomena of autophagy in plat cells is elucidated. Integration of carbon and nitrogen metabolism in roots is discussed with particular emphasis on intracellular compartmentation of processes involved in nitrate assimilation. Control mechanisms, affecting the rate of respiration in roots are discussed, significance of subcellular compartmentation of substrates in transport processes and rate-regulation of respiration is outlined. Topics of partitioning of respiration between maintenance and growth, and relationship between relative growth rate and respiration are also covered. Role of sucrose and adenylates as rate regulators of respiration and their mode of action are discussed. Separate and combined effects of growth regulators, light quality and calcium on respiration rate in the shoots are described.

Z. NAGY

ENCKE, F.—BUCHEIM, G.—SEYBOLD, S.: Zander Handwörterbuch der Pflanzennamen (14th edition). Eugen Ulmer GmbH & Co., Stuttgart 1993, 812 pp.

The first edition of this dictionary was edited by Robert ZANDER (1892—1969) and "Zander" became a wide used dictionary treating several plants by time. So, it is a very useful pocket book for wide range of readers. Its structure also became traditional through the series of revised editions.

After Preface the book contains curriculum vitae of the first editor Robert ZANDER. It is followed by an introduction to botanical nomenclature including the International Rules for Botanical Nomenclature. This part of the chapter is divided to two other parts containing the International Code for Botanical Nomenclature and its application in part a, and the International Code for Nomenclature of Cultivated Plants in the b.

The 2nd chapter deals with the regnum of plants with special regard with plants above of Pteridophyta, while the 3rd one gives an alphabetic list of families and genera. This is set from taxa mentioned in the next part of the book, which one is the main part of the dictionary. One could find a commentary to make easy the use of alphabetical list of genera of plants, then there are explanations of marks used and the whole forms of abbreviations.

The most important part of the 4th chapter and the book is the list of genera. This contains the name of genus, author and the family which it belongs to. The list of species is findable under genera, with correct name of species, authors, and some other important data, like the manner of living in culture and origin of them. There are the widespread synonyms, too.

The further chapters are containing lists of common names of plants, explanations of Latin names and the correct whole name of authors cited in the dictionary. This latter list is very important, because most of the books dealing with plants use the abbreviated forms of the author-names.

However some viewpoints of the dictionary, e.g. the large concept of genus Tanacetum at cost of Chrysanthemum, are disputable, this dictionary is well usable for gardeners especially, but also for other interested readers.

E. I. SIMAY

FACCIOLA, S.: Cornucopia — A Source Book of Edible Plants. Kampong Publications, Vista, Ca., USA 1990, 678 pp.

No one can say exactly how many plant species there are, but scientists estimate their number around 2-300 000. Out of them at least 20000 have usefully edible parts: seeds, tubers, fruits, leaves, etc. But only 3-4000 have ever been used on a regular basis, and fewer than 150 species have been brought into what we would consider substantial "agriculture". Even these are underexploited in terms of their genetically potential, because we focused our attention on very few of them, like cereals, potato, some fruits, etc. Most of the world's food is being produced from these twenty or so, grossly overworked, species.

It is a very narrow base for our food source and too small larder for feeding the mankind. We are just about to realize this vast heritage of ours and at the same time it is being threatened. During the last decades hundreds of species and varieties of plants have been lost — not only from the remote islands and rain forests but also from our gardens.

In the light of these facts, one cannot overestimate the value and importance to such a book. Stephen FACCIOLA's effort was to put together an easy-to-use compendium of the diversity of food plants available to consumer, gardener, plant breeder, and scientist. It includes more than 3000 crop species. Not only higher plants, but also mushrooms and microorganisms are included. It can serve as both a reference or source for information on plants, and a guide to actually finding, growing and using them.

110 major crops have been chosen for detailed cultivar listings representing the most popular fruits, vegetables, grains, herbs, nuts and mushrooms.

Taxonomic nomenclature follows that of TANAKA and KUNKOL. Classification of cultivars is modified after LEWIS and Hortus Third.

The book is divided into three sections: Botanical listings, Cultivar listings and Sources.

For each species, the type of plant material or product, a description of how the plant is used, distribution, annotated bibliographical citations, and sources are given.

For cultivars, the days to maturity, general features, origin, citations, and sources are enumerated.

Included in the Sources chapter are the names, addresses of more than 1350 firms and institutions including 1050 from USA and Canada, 150 from other countries and 150 non-commercial sources (botanical gardens, arboreta, research stations, etc.).

CORNUCOPIA is really a powerful and authoentic reference book and also a useful tool to find the sources. It has been written for botanists, plant breeders, genetic preservations, educational institutions, professional and private gardeners, etc.

I can specially recommend CORNUCOPIA for Central and Eastern European professionals and the interested public for it contains many promises for our future agriculture in terms of increase of the species and variety diversity in our fields and gardens. There are hundreds of potential new crops or new varieties of our well-known crops in this book waiting for their man to take the adventure of try them. Quite surely, many of them would offer a real opportunity on smaller or larger scale to be added to our existing choice of crops and varieties, and also provide new raw materials for food, cosmetic, and other industries, or simply to find a beautiful new flower for your garden.

The book can be purchased directly from Kampong Publicatins (1870 Sunrise Drive, Vista, Ca 92084, USA).

Z. SZŐCS

FRIEDMANN, E. I. (ed.): Antarctic Microbiology. Wiley-Liss, New York 1994, 634 pp.

This book is an excellent summary of our knowledge about the (biology) microbiology of an extreme region, written by 29 contributors. The title of this book can be unusual, therefore FRIEDMANN and THISLE tell us the reason in the foreword: "Why Antarctic microbiology? Why not African, Australian or European microbiology? The answer is that the biology of Antarctica, more than that of any other continent, is dominated by microorganisms. On the continent itself,

plants (except microalgae) are scarce. Higher plants are represented by only two species, Colobanthus crassifolius (d'Urv.) Hook, f. and Deschampsia antarctica Desv., both restricted to the Antarctic Peninsula. Mosses are relatively minor primary producers compared to microalgae and cyanobacteria. Even in the ocean, macroalgae are less significant than in other coastal waters, and the food chain of the rich marine life is based almost entirely on microalgae and cyanobacteria. The nearly total absence of macroscopic terrestrial animal life further contributes to the unique character of the biology of the Antarctic continent. The role of plant cover on land is taken over by lichens, which grow on the surfaces of rocks and soil in the maritime Antarctic and are hidden under the surface of rocks in the more extreme desert regions."

The first group of topics concentrates to the marine environments. There are contributions focused on microbial processes of chemoheterotrophic bacterioplankton populations; horizontal and vertical distribution, seasonal variability and primary production of Antarctic phytoplankton; functional role of pelagic protozoa, classification by their trophic mode and size, abundance, distribution and feeding relationships of protozooplankton; diverse communities of sea-ice biota and their physiological ecology; autotrophic and heterotrophic communities of nearshore benthic marine sediments.

The second group of topics concentrates to the terrestrial and freshwater environments of the Antarctic continent. There are contributions focused on frequency of viable microorganisms at various depths in the Antarctic ice sheets; the microbial abundance and diversity in Antarctic soil and environmental factors controlling the activity of microbial communities; different terrestrial lithophitic microorganism like the lichen-dominated or cyanobacteria-dominated or red Gloeocapsa-dominated etc. cryptoendolithic communities and their life, their role in the Antarctic rock surface zone; lichens living on rock surface, their environmental adaptation and dependency on weathering, nutrient supply; environmental regulation of microbial activity is continental Antarctic lakes; bacterial and algal communities in Antarctic flowing waters.

The third topic' group is variable. There is contribution about human infectious diseases; about exobiology or about protection of Antarctic microbial habitats.

It is obvious that many highly unusual habitats, life strategy, microbial communities exist on Antarctic continent. A lot of new results are shown here and reading the book a lot of questions can draw up for the future studies. This well-illustrated book is undoubtedly a definitive reference work, essential for all microbiologists, algologists, botanists, biologist with a serious research interest in this subject. We recommend it also in university education.

K. T. KISS

FRÖHLICH, G. (ed.): Wörterbücher der Biologie — Phytopathologie und Pflanzenschutz. Gustav Fischer Verlag, Jena 1991, 382 pp. (2nd edition)

This dictionary has appeared as No. 867 of Uni-Taschenbücher series. Its main purpose is to give explanations for wide range of terms in very different aspects of phytopathology and pest or disease controlling methods. Because the scientific advance of phytophathology resulted a mainly specific terminology for it this pocket-book is very usable.

We could find expressions dealing with wide range subjects connecting with phytopathology. These include terms of acarology, mycology, nematology, virology, etc., but also the different aspects of control. 104 inserted figures are demonstrating organisms, tools and scheme of processes. Some morphological marks are also illustrated like antens of insects, fruiting bodies of fungi or sexual dimorphism.

Subjects of controlling are included symptomatology and diagnosis of diseases, aspects of resistance to pests and diseases, epidemiology, methods, etc. This edition deals with biological control, too, e.g. biotest, biopreparates, parasitism including hyperparasitism, parasitoids.

By the wide ranges of mentioned subjects this book, pocket dictionary is well-usable for readers working in agriculture or studying this science.

E. I. SIMAY

GROVES, R. H.—DI CASTRI, F. (eds): Biogeography of Mediterranean Invasions. Cambridge University Press 1991, 485 pp.

The problems of biological invasion have recently received considerable interest all over the world. The idea for this volume originated in 1983 at the first meeting of the Scientific Advisory Committee for the program on the Ecology of Biological Invasions. This book is the part of the impressive publication lists on the subjects of mediterranean ecology and of biological invasions presented during the last decade.

The book comprises 31 chapters.

In the introductory interview a clustering of the regions of mediterranean climate into "similarity complexes" according to some geographical and ecological characteristics is given by DI CASTRI. Similarities among the 5 regions due mostly to the geological and topographical features, evolutionary convergence patterns, phylogenetic commonalities and parallel human impacts are presented, as well as some factors that appear to play a major role in explaining the processes of biological invasions and to contribute to the relative vulnerability (invasibility) or resistance against invasion of the 5 regions are also emphasized.

After the introduction, 5 chapters deal with the historical background to invasions in all 5 mediterranean-climate regions, especially as that background relates to human settlement of them. The following 10 chapters on biogeography of higher plant taxa cover different topics: 5 of them mainly refer to the introduced flora of the different regions and the history and the rate of success of invasions and 5 of them describe ecological aspects of some well-documented plant invasions. The chapter written by OLIVIERI et al. extends the discussion of life cycles of some mediterranean invasive plants which show diverse life history traits in relation to their biography and their invasive abilities. Specht's chapter addresses the relationships between plant invasion and soil seed bank and Lepart's chapter deals with the invasion processes as related to succession and disturbance.

The problems of invasiveness of mammals are concerned in the following 5 chapters. Current distribution and speciation among mediterranean mammals, faunal exchange between Europe and Africa and introduced mammals in California, in mediterranean region of South Africa and in southern Australia are discussed in detail. The subsequent 4 papers deal with invasions and range modifications of birds in the 5 Mediterranean-climate regions especially as a result of human-induced changes.

Finally, 4 chapters approach the applied aspects of mediterranean invasions. They cover both the topics of weed invasions in agricultural areas, plant invasions in the rangelands, consequences of forest plantations on the process of invasion and the importance of mediterranean-adapted dung-beetles (Scarabidae). In these papers the nature and causes of invasions and the ecology of invasive organisms are mostly discussed in a more applied sense in relation to the control of biological invasions and to the required land use.

In the 2 overview chapters Groves gives some generalisations about the biogeography of plant invasions in mediterranean-climate regions and DI CASTRI makes some preliminary observations and explanations concerning animal invasions in general.

In summary this volume provides many examples and presents useful up-to-date reviews on the process of invasion by plants and animals and the invasiveness of some ecosystems in the 5 regions of mediterranean-climate by showing many interesting results. Its special adventage is that the case studies in the book represent a lot of raising unsolved difficult questions and a good material to promote the development of further investigations concerning of the ecology of biological invasions.

K. VIRÁGH

HAWES, C. R.—COLEMAN, J. O. D.—EVANS, D. E. (eds): Endocytosis, Exocytosis and Vesicle Traffic in Plants. Cambridge University Press, Cambridge, New York, Port Chester, Melbourne, Sydney 1991, 252 pp.

This book is the 45th volume of the Society for Experimental Biology seminar series. The chapters are based on papers presented at a symposium held by the Society for Experimental Biology Annual Meeting at the University of Warwick, 1990.

The vesicle traffic and transport of the animal cell is a thoroughly investigated area of cell biology, much less is known about these topics concerning with the plant cells. This book provides deep insight in this field based on numerous experimental data and observations.

The first part of this volume gives a general theoretical approach of vesicle traffic involved in exocytosis and endocytosis using electronmicroscopic, biochemical and genetical results as well. One of the most remarkable topic is the description of two distinct endocytotic pathways in plant cells, linked the plasma membrane recycling and the degradation of molecules carried by coated vesicles to vacuole.

Two chapters show the fine structure, isolation methods and enzymic characteris of coated vesicles.

For the first sight the chapter written by P. L. STEPONKUS is in not close connection with the subject of the book. His studies were directed to an understanding of the mechanismus of freezing injury and the effect of cold acclimation. The results however provide insight into the membrane structure and mechanics, and it is evident that these characters are strictly involved in exo- and endocytosis.

The second part of the book indicates link between vesicle transport and some physiological events of plant cells.

The results of STEER and O'DRISCOLL clarify the importance of vesicle dynamic and cell membrane turnover in cell growth. The mechanism of protein secretion is illustrated by hidrolase secretion of cereal aleuron grains.

The next topic focuses the secretory pathway of cell wall components and its connection with Golgi activity and vesicle transport. The chapter is illustrated by photos of high quality and expressing drowing. This latter gives a model on synthesis, transport and excretion of cell wall polysaccharides.

The most complex mode of plant secretion is the exocytosis in gland cells. KRONENSTEDT-ROBARTS and ROBARTS provide an excellet summary of this topic based on former works and completed by numerous up-to-date informations.

The editors point out in the preface, the authors of chapters were given the possibility to present their work in original form, without any change in the manuscripts. This results in some cases overlapping of chapters but it does not mean the drawback of the book, rather improves the complex approach of its subject.

The book is recommended to the advanced students of universities, and experts on every field in biology who could get valuable informations on transport of macromolecules in membrane-bounded vesicles of plant cells.

E. MIHALIK

HAWKSWORTH, D. L. (ed.): The Biodiversity of Microorganisms and Invertebrates: Its Role in Sustainable Agriculture. C.A.B. International, Wallingford 1991, 302 pp.

Maintenance of biodiversity has a very high importance for sustainable agricultural production and environmental protection. However the importance of microorganisms and invertebrates in the stable functioning of ecosystems has attracted less overt attention. So, a workshop was organised by CAB International in association with the Committee on the Application of Science to Agriculture, Forestry and Aquaculture (CASAFA) of the International Council of Scientific Unions, the Commonwealth Science Council, and the Third World Academy of Sciences for discussing that. Review papers and discussions of this workshop are covered in this book published in CASAFA Report Series under No. 4.

Part I of the book covers papers dealing with importance of microorganisms and invertebrates in the conservation of biological resources, and the function and management of agricultural and natural ecosystems. This part contains five chapters and general discussion of session. Importance of organisms mentioned to sustainable agriculture and as components of biodiversity is discussed in Chapters 1 and 2 by W. D. P. STEWART and R. OLEMBO. D. L. HAWKSWORTH and L. A. MOUND inform about role and problems of biodiversigy databases in Chapter 3, while J. BOUSSIENGUET deals with problems of assessment of biodiversity in Chapter 4. The profile of insect biodiversity is reviewed in Chapter 5 by J. D. HOLLOWAY and N. E. STORK with

particular attention to the use of invertebrates as environmental indicators. Chapter 6 is the general discussion of Session I, where M. H. was the chairman.

Part II contains informations about connections of biodiversity and sustaining of soil productivity. The biodiversity of soils is considered by K. E. LEE in Chapter 7, particularly in relation to the larger invertebrates. In Chapter 8 R. LAL reviews the soil conservation including agricultural practices that enhance the soil activity and diversity of the soil biota might be reducing risks of runoff and soil erosion. I. SZABOLCS's contribution in the next chapter deals with the properties and biodiversity of salt-affected soils by thier five groups, and the possibilities for thier utilization, too. P. A. ROGER, K. L. HEONG and P. S. TENG discuss the role and potential of invertebrates and microorganisms in biodiversity and sustainability of wetland rice production in Chapter 10. The authors state that increasing or preserving diversity per se needs developing effective trophic linkage. Chapter 11 is also a discussion of whole Session by J. M. LYNCH and J. K. WAAGE.

Next Session papers in Part III discuss the role of biodiversity in pest occurrence and management. Aspects of biodiversity in tropical pest management and increasing biodiversity to improve pest management in agro-ecosystems are presented in Chapters 12 and 14 by T. J. PERFECT and M. A. ALTIERI. This part also deals with the possibilities of biological control, and J. K. WAAGE's paper (Chapter 13) treats the biodiversity as a resource for biological control. M. F. CLARIDGE in Chapter 15 presents the connections between genetic and biological diversity of insect pests and their natural enemies, pointing out the necessary of taxonomic works, too. General discussion of the Session is given by G. H. L. ROTHSCHILD in Chapter 16.

Papers comprising biotechnology aspects are set in Part IV. A. T. BULL (Chapter 17) describes the benefits to be gained from molecular biology research and the many opportunities that can arise from genetic engineering. Attention to mixed symbiotic cultures is also drawn. Paper presented by J. E. BERINGER, P. K. HAYES and C. M. LAZARUS in Chapter 18 treats the methodology aspects of culture collections and also describes the benefits of storing DNA sequences in computers. Importance of biodiversity to biotechnology is presented by L. J. NISBET and F. M. FOX in Chapter 19. This chapter reviews the many industrial applications of microorganisms including wide range of them from which useful products can be obtained. In the next chapter P. R. SCOTT directs attention to importance of information transfer. The need for consistent names and/or codes for organisms is also reviewed. The Session-discussion is given by J. H. HULSE and D. L. HAWKSWORTH.

Because of the complex themes of the book and its valuable papers, this publication is well usable for wide range of readers.

E. I. SIMAY

INGWERSEN, W.: The Alpines. Sagapress/Timber Press Portland, Oregon, John Murray (Publishers) Ltd. 1991, 308 pp., 350 colour photographs

Will INGWERSEN (died 1990) grew and indeed sold alpines from his earliest childhood. In the course of a long life (he knew both William ROBINSON and Lawrence JOHNSTON) he held high office in the Royal Horticultural Society, the Alpine Garden Society and elsewhere, and among many award he received the Victoria Medal of Honour.

The book helps alpine gardeners to find the alpine plants to be planted for all season. From the early spring when the glory of the rock garden is said to be it shows the alpine plants opening their flowers throughout the year. There is a whole chapter dedicated to the many shades of the blue flowers appearing from the spring like bluebells till the autumn when a final fling is provided by the brilliant gentians. Some of the Campanulales and their relations are described as the cream of the genus as far as the rock garden or alpine house concerned. In alpine gardening one is so used to dealing with genera which contains large number of species that it comes as something as a surprise to realize how many consist of only one member. There are a selection of these interesting group of plants in a separate chapter.

Primeroses the vast genus of Primula is occupying a great many of the Alpine's pages. There are more than a hundred species spread around the north temperate zone. It contains Transatlantic alpines from North America and a whole chapter is given to Lewissias and to their

relatives. There are a few poppies (Papaveraceae) that can claim admission to the rock garden but that those that do qualify are extremely desirable and are included in this mannual. Although woodlanders are far from restricted to tight cushion-forming species of the high mountains many alpine gardeners grow a wide range of them. A few pages help the gardeners enjoy these plants coming from much lower altitudes.

The Iris family (Iridaceae) is a large one including several species loved by the alpine gardeners. There are very practical chapters like the "Weeds and well-behaved" which gives a hand to the gardener to select the well-behaved plants from the weeds which might belong to the same genera sometimes. Dwarf shrubs are an important element in the rock garden. They not only give it texture and structure, but also provide a serious of microclimates in which other plants can grow, and shade cast is particularly important. There are separate chapters for Caryophillaceae, Compositae, Ericaceae, Ceraniceae, Ranunculus, Rosaceae and for the Viola genus.

In recent years there had been a great interest in the alpine flora of New Zealand. There is a section containing these plants thriving in the rock garden transplanted to the northern hemisphere. Many books of rock garden plants leave out bulbs but here one can find a satisfactory range these plants including Cyclamen, Tulipa, Narcissus, Colchicum, Hyacinthoides, Ornithogalum, Allium, Fritillaria, Pleione genuses.

Many of the flowering plants put out their display in the spring and foliage helps sustain interest for the rest of the year. As the foliage effects play an important part of the appearance of the rock garden, it got an own chapter. One can easily get practical advise how to grow succulents, or alpines with a fragrance, whether to put them in a hot or a shady place to make them grow better or not.

Although this book intends not to be comprehensive it is useful enough for those who grow alpines and those who simply love rock garden plants. Many of the illustrating photographs were taken by Peter STILES and make the book invaluable for everyone.

R. GOLDMAN

JEFFREY, D. W.—MADDEN, B. (eds): Bioindicators and Environmental Management. Academic Press, London, San Diego, New York, Boston, Sydney, Tokyo, Toronto 1991, 458 pp.

This book is a collection of materials presented at the 6th International Bioindicators Symposium held in Trinity College, Dublin, 23—28th September 1990. The volume consists of 35 papers. The aim of the symposium was "to promote the transfer of ideas regarding potential bioindicators, and originating in laboratories, into the harsher realities of filed environmental monitoring". This effort has great importance because as in the introduction of the book it was mentioned that "although the concept of biomonitoring is ancient, its application to current monitoring problems is relatively slow to develop".

Papers dealing with problems of different ecosystems in several aspects were arranged under four subtitles.

The first one is "Bioindicators, Industry and Administration". This part contains 9 papers. Most of them are monitoring studies carried out in aquatic environment (marine, estuarine, freshwater) suffering from industrial pollution. There is an article in this chapter, which deals with an interesting, current theme; monitoring the effects of agricultural pesticides on wildlife.

In the second part of the book we can get information about "Environmental Radioactivity and Biomonitoring of the Chernobyl Accident" on the basis of 3 papers.

Under the third subtitle — Monitoring Long Term and Large Scale Environmental Trends — 5 papers can be read. Using the structure of animal communities, presence, abundance and distribution patterns of sensitive indicator plants as bioindicators of environmental changes were discussed. Provision for Areas of Outstanding Natural Beauty was also mentioned in this part.

The last chapter deals with "Basic Research in Biomonitoring". This is the most extensive part of the book. Animal behaviour, productivity, physiological, enzyme activity changes

of test organisms can also be used to indicate environmental pollutants. Information about recent developments on this field can be available in more than dozen papers of this part.

This book is very useful for scientist working in environment and nature conservation. In the great variety of papers included in this book everybody can find valuable, interesting particulars relating to his own research field.

B. PAPP

KRAMMER, K.: Pinnularia, eine Monographie der europäischen Taxa. Bibliotheca Diatomologica. J. Cramer, Berlin—Stuttgart 1992, 353 pp.

This volume of the excellent book-series is focused on one of the most important genus of pennate diatoms. Since the first revision of Pinnularia, published about hundred years ago (P. T. CLEVE 1895) a lot of species was newly described and added to this genus. Therefore it was very much on the agenda to write a monographic book of European taxa.

Up to now, 949 correctly described taxa have been published (more than 700 in this century). Unfortunately many taxa (more than two hundred) were described on the basis of one specimen. Unfortunately many authors did not take division cycle into consideration, when larger or smaller specimens have numerous convergencies and parallelisms with other taxa. Therefore description or typification of taxa on the basis of a single specimen is not almost suitable.

"Additionally, the valve taxonomy of the diatoms has special problems, different from higher plants and algae, one must base a modern diatom taxonomy on the examination of the division cycles from the auxospore to the gametes and the changes of the outlines and other important characteristics. Unlike when dealing with other plants and animals, the examination of the variability of the valves in different populations is necessary" — is written in the foreword.

The author starts with the general concept of diatom systematic, by the systematical bases used during his study. It is discussed also the effect of life cycle on diatom taxonomy; than general characteristics of Pinnularia genus. The fourth chapter starts with the key of species and continues with description of 96 species known from Europe. By description after detailed morphological characterisation of each species, there are data about their occurrence, dominancy, abundancy and ecology. There are discussed "only" 96 Pinnularia species, but with their subtaxa. Therefore a few hundred taxa are described in this book (e.g. under P. borealis 9 varieties are discussed and many of them were synonyms of former species or varieties). The chapter finish by Latin diagnosis of 36 newly described species or varieties. Determination of Pinnularia taxa is more easy by 76 Table of Figures (contain more then thousand excellent LM micrographs and few SEM ones).

It was a very good and useful idea for the English speaking users of this book to complete the introductory chapter with an English key of Pinnularia species.

We can recommend this excellent monograph from a difficult and complex group of pennate, to algologists, applied hydrobiologists and also for basic systematic work and university education. This book is indispensable for all specialists working with diatoms of lakes and rivers.

K. T. KISS

LANGE-BERTALOT, H.: 85 Neue Taxa und über 100 weitere neu definierte Taxa ergänzend zur Süsswasserflora von Mitteleuropa. Vols 2/1—4. Bibliotheca Diatomoligica 27. J. Cramer. Gebrüder Borntraeger, Stuttgart 1993, 454 pp.

85 new species, subspecies, varieties and forma are described in this book. More than 250 taxa from Europe and elsewhere are discussed here. There are also a few additional "Sippen" of questionable identity and status introduced provisionally. All of these taxa belong to 30 genera some of which are fruits of recent taxonomic splitting. The author did not create

any new genus, but there is a few new subgenus descriptions and he hopes they will not introduce more instability into the interim but well-established nomenclature system.

LANGE-BERTALOT wrote in the abstract and foreword of the book the following: "This is a book written from the critical point of view of a practitioner using diatoms chiefly in applied hydrobiology. It is intended as a supplement to Vols 2/1—4 of the 'Süsswasserflora von Mitteleuropa'. Established taxa in literature are re-examined in light of the realities in nature and inconsistencies in the literature are exposed. Some findings may be puzzling for novices in floristic diatomology; this demonstrates that the reality is not as simple as suggested in many earlier and recent publications."

"A supplementary volume to the just completed 'Freshwater Flora' with its 2500 pages in 4 volumes dedicated to diatoms? The question as to why is it necessary is justified. The simplest answer is that this flora will never be 'complete' even if restricted to just Europe or even Central Europe. Intensive, critical study of historically documented taxa has led to a relatively advanced preliminary state of knowledge. The preliminary state was presented as we saw it. Without having taken the risk of documenting fragmentary knowledge, hydrobiologists interested in diatoms as indicator species would have had to wait quite a long time for an up-to-date taxonomical foundation. Building upon this foundation, and having shed ourselves of the largely vague and contradictory concepts of taxonomic identity, much progress has been made in the short time since completion of the 'Freshwater Flora'. The photographic documentation proved itself in an outstanding way and reveals the essence much better than subjective and abstracts drawings despite a few shortcomings."

We think this "supplementary volume" of Süsswasserflora von Mitteleuropa is an important and useful book of diatom taxonomy. The author is one of the best expert in the taxonomic science and first of all in the taxonomy of Pennales species. Taxa discussed in this book belong 25 genera. A detailed summary can be found before discussing each taxa or taxon-group. Description of new taxa, new combinations (nov. comb. nov. stat.) are completed with light- and/or electronmicrographs. 1327 micrographs are found on 134 plates. Due to a printer's error the plates of the volume were reduced to 90%. Therefore a supplement volume was published with all plates in the correct size, unreduced.

We can recommend this well-illustrated manual for algologists who wish to develop their skills in identifying freshwater diatom species. It can be essential for algologists, botanists, biologists as a new complementary book of "Süsswasserflora von Mitteleuropa" series. We recommend it also in university education.

K. T. KISS

MASALLES, R. M.: Flora i Vegetacio de la Conca de Barbera. Institude d'Estudis Catalans, Barcelona 1983, 232 pp.

This book presents the results of a five year study, regarding the vegetation of Conca de Barbera, a Catalan county. The book rightly deserved the Pius Font i Quer prize, that was founded for the memory of the great Catalan botanist. It was written in Catalan, but scientists who know Spanish or French, will understand the text, which uses simple expressions and frases.

The book is actually a flora catalogue for this county, which is situated in the centre of Catalonia. The configurations of its terrain is mixed. The introduction is a brief description of the county's environment. A large part of the county is fertile river valley or basin, suitable for agricultural cultivation, interrupted with small chains of mountains. Rocky mountains lie at the border of the county. The climate is typically mediterranean, though small climatic variations occur according to the topography. Limy marl and conglomerates are frequent at the low areas, while the base rock of the South mountains is granite. The vegetation is varied according to the local variations of climate and base rock. It consists of mainly Mediterranean elements, but Eurosiberian and Atlantic species can also be found (e.g. Prades mountain).

Agricultural production is intensive here for centuries. 45% of the area is still under cultivation. Mainly corn and vine is grown, but olive plantations and orchards cover large areas, too. As the county was left out from the industrial development, many of the

inhabitants move and the area of abandoned fields is growing. The study of vegetation dynamics on the meadow communities that appear on the abandoned fields has a great emphasis in the book. Naturally we can find short descriptions of other meadow communities present at the area, also.

Besides the herbarium data collected mainly from the NE and SW parts of the county, the author also used data of his own investigations about the inner part, which area was not explored botanically so far. The precise data of locations for the almost 1200 species listed in this catalogue, are given by the codes of the UTM screens. There are complementary data presented regarding the ecology of the species' habitats.

The book provides lots of useful information for botanists working on sub-mediterranean and mediterranean areas.

B. PAPP

MELKONIAN, M. (ed.): Algal Cell Motility. Current Phycology 3. Chapman and Hall, New York and London, 236 pp.

This book is a good summary of our knowledge about the motility of algae, written by 11 specialists of subject. "Algae exhibit the greatest variety of cell motility phenomena in the living world. These range from the peculiar gliding motility of filamentous blue-green algae or cyanobacteria to chloroplast movements and cytoplasmic streaming which are most common in higher plants. In addition, cell motility by eukaryotic flagella is the characteristic mode of cell locomotion in algal flagellates and most reproductive cells of algae" — can we read in the preface.

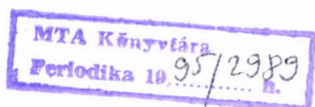
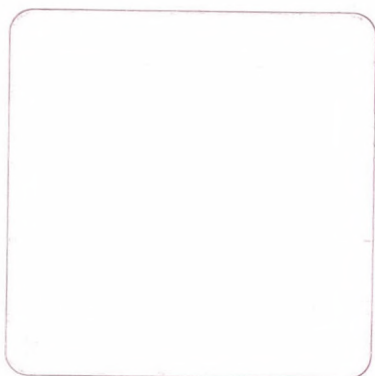
One of the most mysterious movements of algal cell is the gliding motility. A lot of detail is quite well known about this kind of motility but we do not know exactly the basic of whole phenomena. There are two hypotheses about the mechanism of gliding motility at Prokaryotes: i. Contractile filaments produce surface undulation that operate against the surrounding slime tube; ii. Slime ejection and swelling cause the surface undulations. Contractile filaments were observed at diatoms too and there are a lot of species making quick and complicated movements.

Organelle movement is a ubiquitous phenomenon of eukaryotic cells with an intact cytoskeleton. The algal chloroplast movements is shown in chapter 2 and 3 by filamentous or thallusous species like Vaucheria, Briopsis, Caulerpa, Acetabularia, Mougeotia or Characean species.

The flagellar beat pattern (Chapter 4) is different, depending on flagella's length (equal or unequal) or beating dynamic (homodynamic or heterodynamic). Description of different beat pattern is discussed showing that of Chrysophyceae, Synurophyceae, Haptophyceae, Xanthophyceae, Eustigmatophyceae, Bacillariophyceae, Dinophyceae, Phaeophyceae, Cryptophyceae, Chloromonadophyceae, Euglenophyceae and Chlorophyceae. Molecular mechanisms of flagellar movements are regulated by calcium, flagellar activity through phosphyorylation (Chapter 5).

In last chapter the centrin-mediated cell motility is discussed in different groups of algae (Chlorophyta, Euglenophyceae, Dinophyceae, Chromophyta, Glaucocystophyta, Cryptophyta and Rhodophyta). A new type of cell motility mechanism has been discovered in the algae, characterised by rapid contractions of an intracellular filamentous structure within less than 20 ms and by much slower reextension of this structure to its original length. Contraction is calcium induced, but independent of external ATP, reextension requires removal of calcium from the structure and is energy dependent.

It is obvious that many highly unusual cell motility phenomena exist in the algae that await a detailed analysis using the now available repertoire of cell and molecular techniques. This book is undoubtedly a definitive reference work, essential for all algologists, botanists, biologist with a serious research interest in this subject. We recommend it also in university education.

K. T. KISS

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1. Title, name of the author(s), affiliation, dataline, abstract, keywords
2. Text, acknowledgements
3. References
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Examples:

Jakucs, P. 1961: Die Phytozönologischen Verhältnisse der Flaumeichen-Buschwälder Südostmitteleuropas. Akadémiai Kiadó, Budapest.

Fekete, G., Précsényi, I. 1981: Niche structure of a perennial sandy grassland. In: Stefanovits, P., Berczik, Á., Fekete, G., Seidl, M. (eds): Man and the Biosphere Programme. Survey of 10 Years Activity in Hungary. Budapest, 68–102.

Borhidi, A., Muñiz, O., Del Risco, E. 1979*a*: Clasificación fitocenologica de la vegetación de Cuba. Acta Bot. Hung. **25:** 263–301.

Jakucs, P. 1973: „Síkfőkút Projekt". Egy tölgyes ökoszisztéma környezetbiológiai kutatása a bioszféra program keretein belül (Síkfőkút-Project. Environmental-biological research of an oakwood ecosystem within the framework of the Biosphere program). MTA Biol. Oszt. Közl. **16:** 11–25.

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